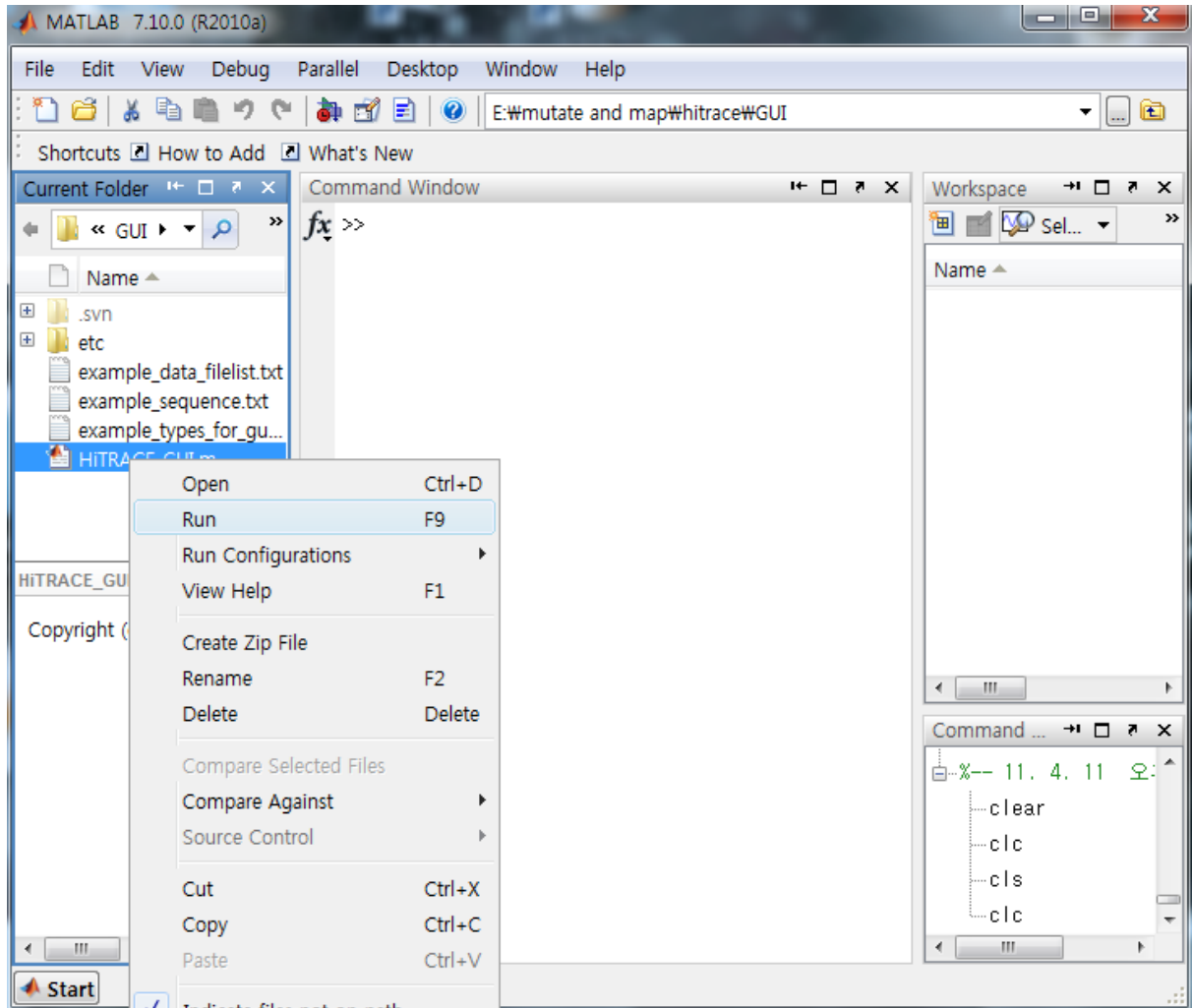


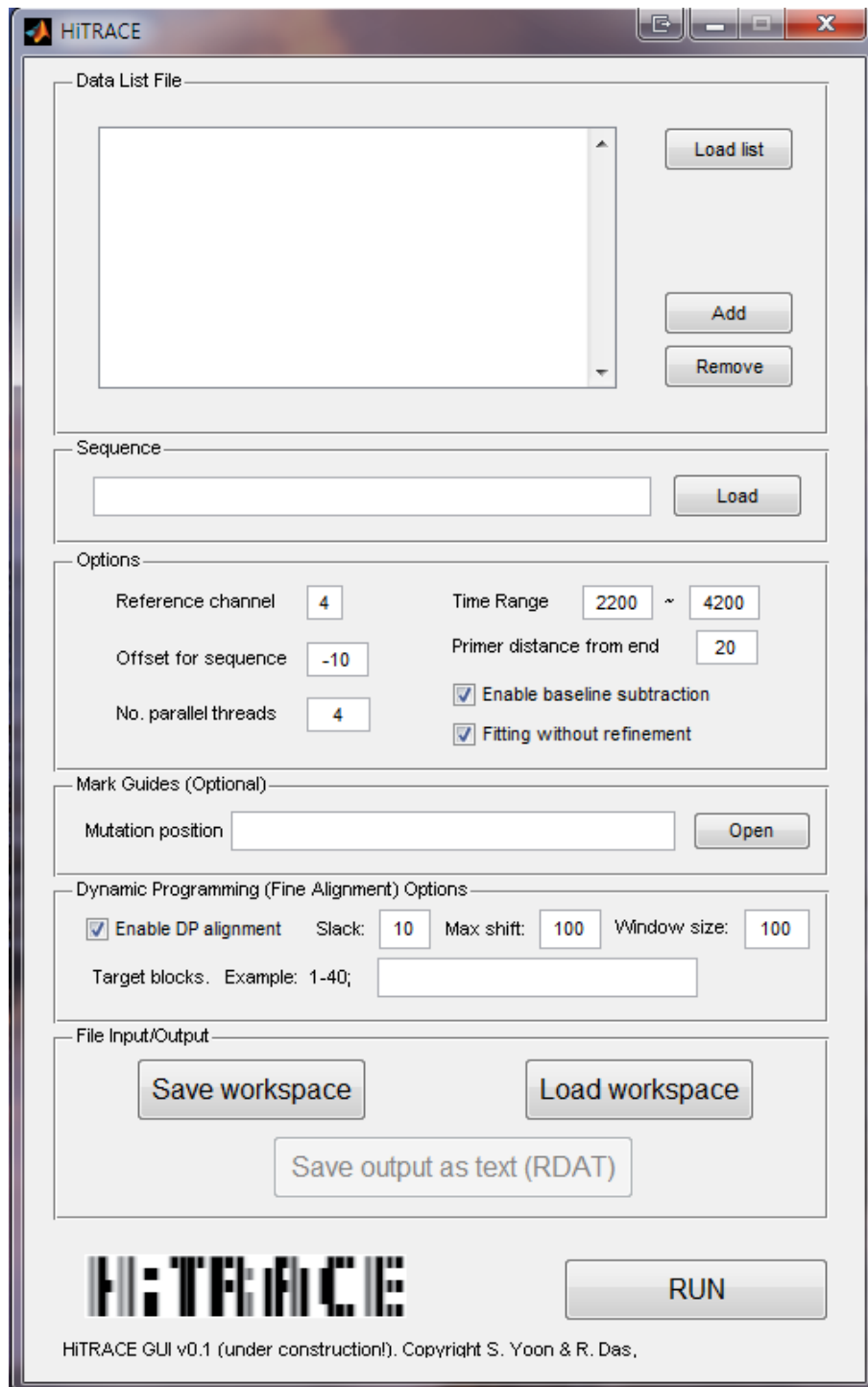
## HiTRACE Tutorial (version 0.1)

### 1. Running HiTRACE GUI



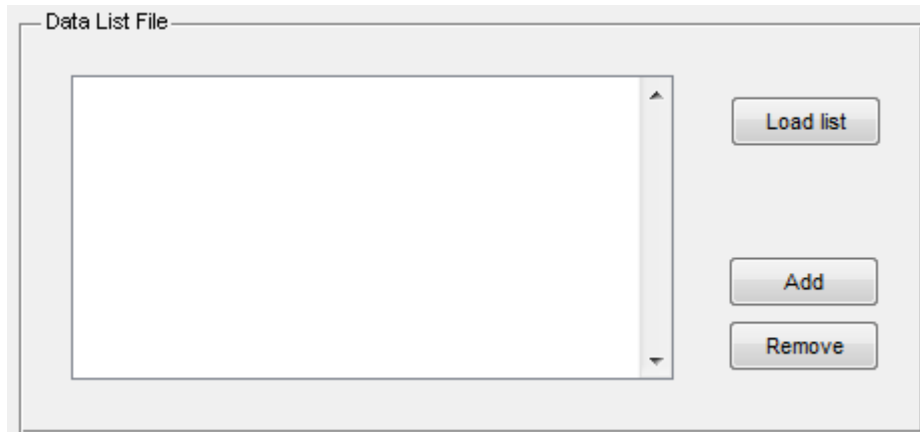
In MATLAB environment, you can launch HiTRACE GUI by right-clicking HiTRACE\_GUI.m in the 'Current Folder' panel and then clicking 'Run.' You can also type HiTRACE\_GUI in the command line and enter. Alternatively, in Windows Explorer, you can right-click HiTRACE\_GUI.m and then click 'Run.'

You will then see the main HiTRACE GUI window:



## 2. Loading input data (required)

You must specify the location of data files in order to start HiTRACE analysis. There are two ways to load input data file(s).

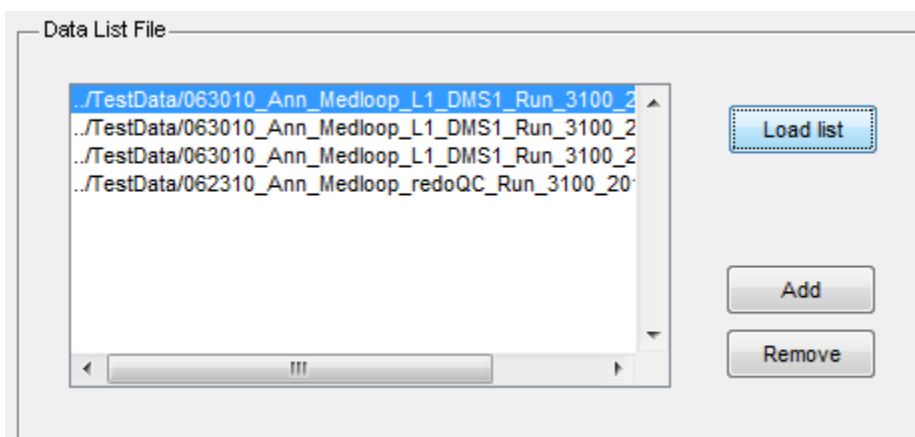


### a. Option 1: Loading data from a file list

In 'Data List File' field of the GUI window, click 'Load list' button and choose a text file that contains a list of folders (one path per line). In this document, we assume that the user selects 'example\_data\_filelist.txt' file, which is included in the distribution.

### b. Option 2: Directly specifying file location

In 'Data List File' field of the GUI window, click 'Add' button and then select the location of a data folder. You can use 'Remove' button to remove a file from the input list. By using a Ctrl or Shift key together with mouse click, you can select multiple files for removal.



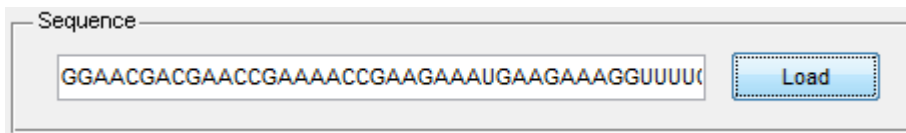
### 3. Loading input sequence (required)

The user also has to load the input sequence. You can either type a sequence string directly in the 'Sequence' window or load one from a plain text file.



The screenshot shows a window titled 'Sequence'. Inside, there is a large, empty rectangular text input field. To the right of the input field is a button labeled 'Load'.

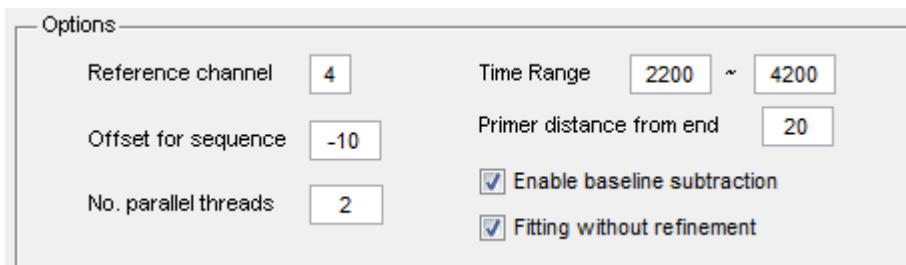
In this document, we assume that the user loads the 'example\_sequence.txt' file, which is included in the distribution.



The screenshot shows the same 'Sequence' window. The text input field now contains the sequence string 'GGAACGACGAACCGAAAACCGAAGAAAUGAAGAAAGGUUUU'. The 'Load' button is still present to the right.

### 4. Adjusting analysis parameters (optional)

There are various options you can specify to fine-tune your HiTRACE analysis.



The screenshot shows a window titled 'Options'. It contains several settings:

- Reference channel: 4
- Offset for sequence: -10
- No. parallel threads: 2
- Time Range: 2200 ~ 4200
- Primer distance from end: 20
- ☒ Enable baseline subtraction
- ☒ Fitting without refinement

#### a. Reference channel

Specify the reference channel index (1, 2, 3, or 4). The default is 4.

#### b. Time Range

The default range is 2200~4200. (The result shown later in this document was obtained with using 1500~4500.)

#### c. Offset for sequence

The default offset is -10.

d. Primer distance from

The default is 20.

e. Enable baseline subtraction

By default, the baseline correction procedure is on, but the user can turn it off by unchecking the box.

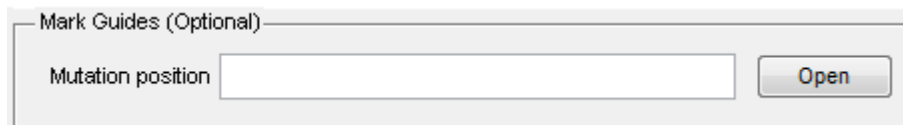
f. Fitting without refinement

By default, HiTRACE carries out fitting without refinement. You can turn on the refinement feature by unchecking the box.

g. No. parallel threads

The number of available threads is automatically detected and displayed. You can adjust the number of threads by entering the number of parallel threads here.

## 5. Marking guides (optional)

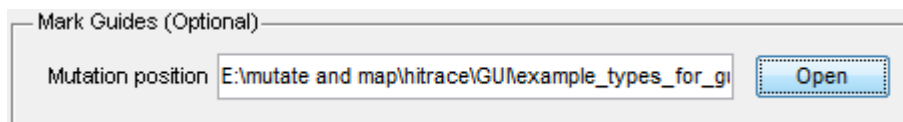


Mark Guides (Optional)

Mutation position

Open

You can show the mutation position by loading mark guides in the interactive manual annotation step. In this document, we assume that the user loaded the ‘example\_types\_for\_guidemarks.txt’ file, which is included in the distribution.

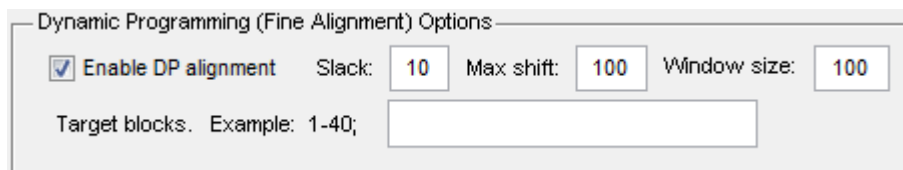


Mark Guides (Optional)

Mutation position

Open

## 6. Adjusting dynamic programming (fine alignment) (optional)



Dynamic Programming (Fine Alignment) Options

☒ Enable DP alignment    Slack:     Max shift:     Window size:

Target blocks. Example: 1-40;

- a. Enable DP alignment

By default, the dynamic programming (DP) based fine alignment is on. You can turn this feature off by unchecking the box.

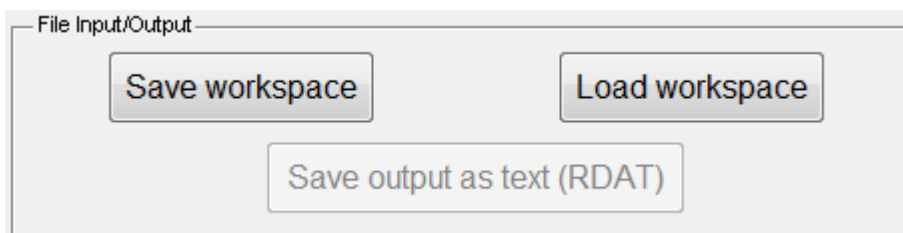
- b. Slack, Max shift, Window size

The definitions of these parameters are provided in the caption for Figure 2 in the supplement. Their default values are 10, 100 and 100, respectively.

- c. Target blocks

By default, the DP-based alignment targets all the blocks. You can select the target block by specifying the range here.

## 7. File Input/Output



By clicking 'Save workspace' button and selecting the location, you can save your workspace. Later, you can load the saved workspace by clicking 'Load workspace' button.

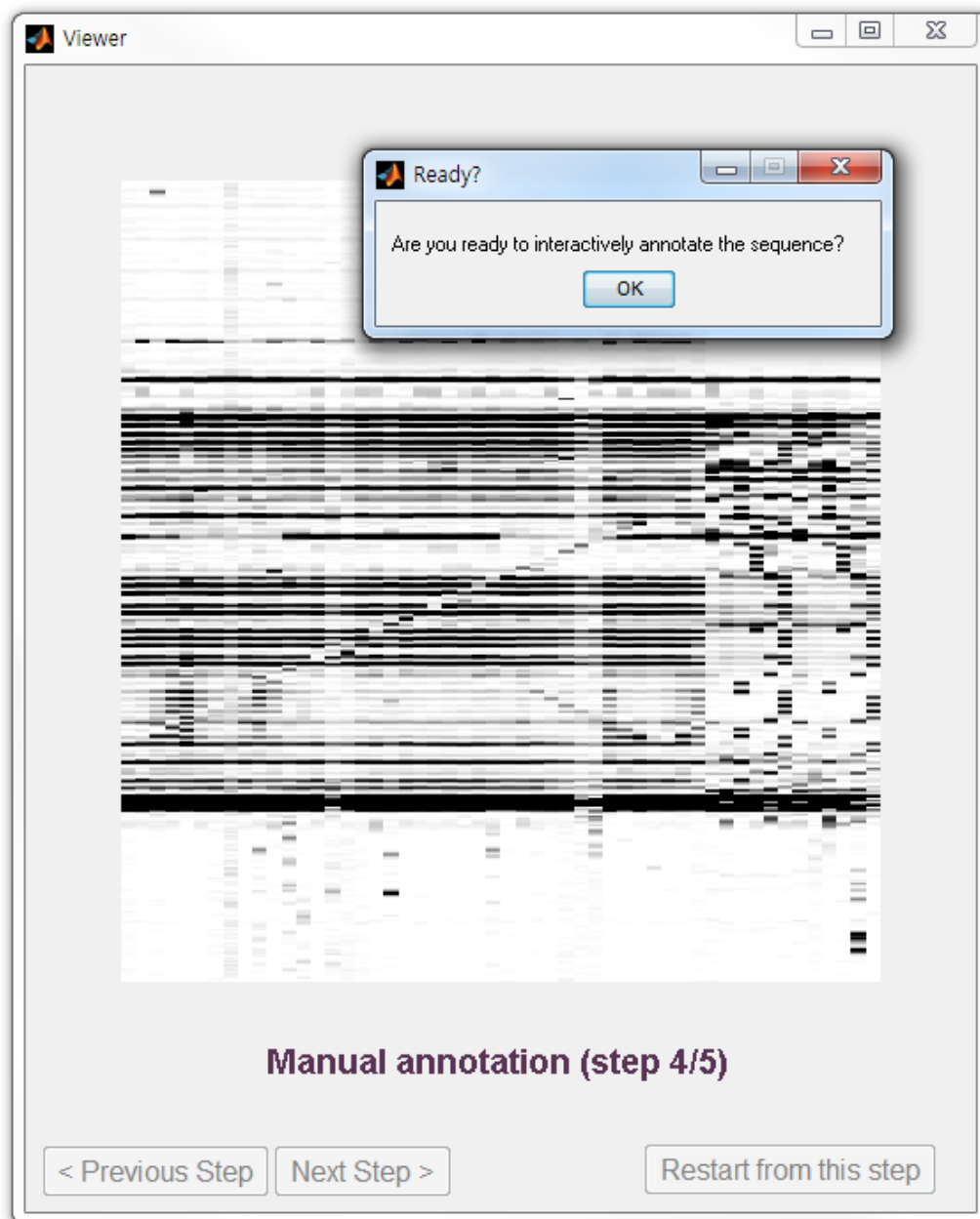
'Save output text (RDAT)' button is to save the final output (quantitated peak areas) of HiTRACE in the rdat format (<http://rmdb.stanford.edu/repository/specs/>).

## 8. Starting analysis



After all the input data and options are specified, you can start HiTRACE analysis by clicking 'Run' button. Note that the label for the button will be changed to 'Update parameters' after the initial run.

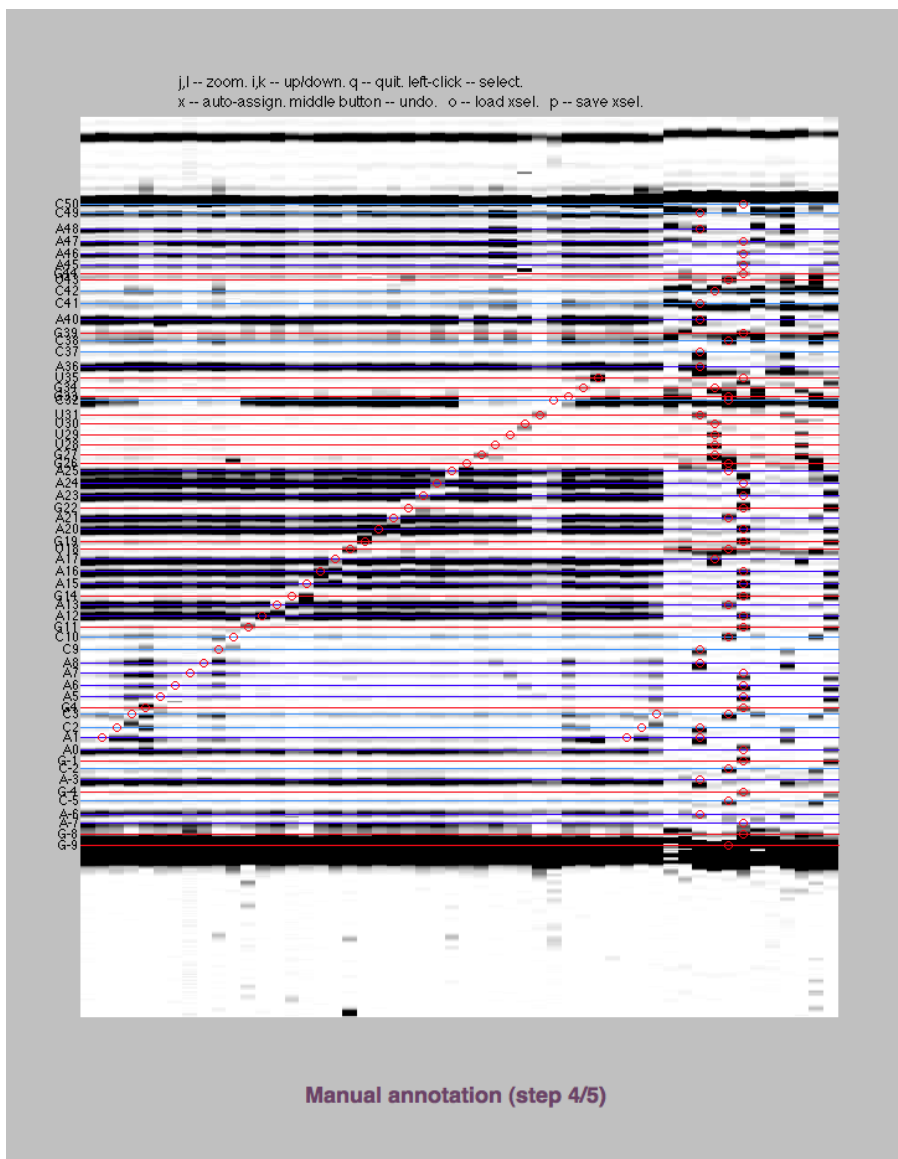
## 9. Manual annotation during analysis



After clicking the 'Run' button explained in the previous section, you will be able to see HiTRACE analysis working. The analysis will wait for your input in Step 4, where you will see a dialog window 'Are you ready to interactively annotate the sequences?' Clicking OK will show the window for manual annotation. In this window, you will have a chance to annotate the peak location manually.

For convenience, there are several short-cuts provided in this step, as summarized below.

Key	Description
j/l	zoom in/out
i/k	up/down
q	quit
Left click	select
x	auto-assign
Middle button	undo
o/p	load/save xsel
1/2	Adjust contrast





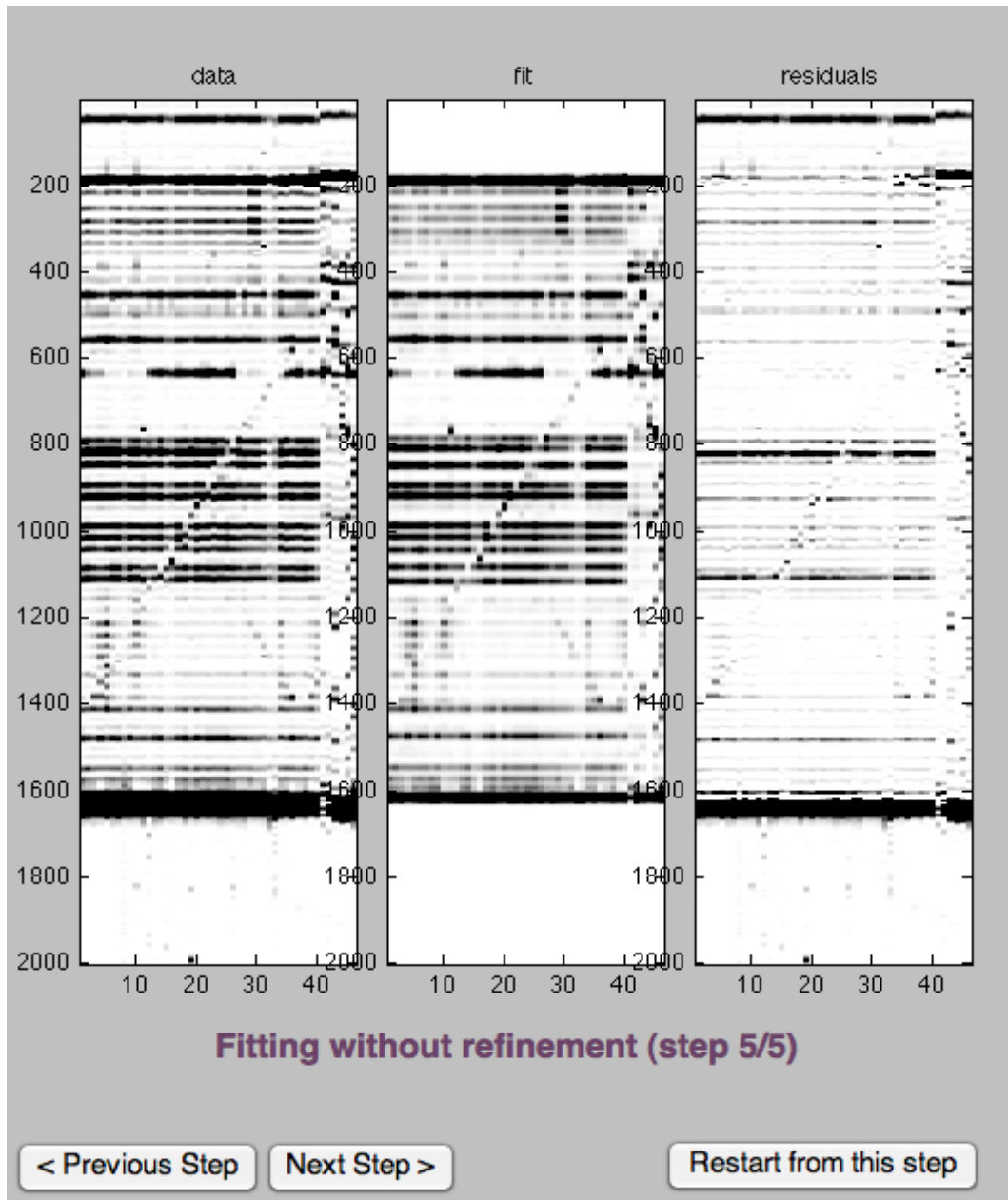
You can select the peak location by a left click. If the user specifies the locations of the first and last peaks and then clicks 'x', the locations of peaks between the first and last are automatically assigned. You can also save the peak location in a file by clicking 'p' and later load it by clicking 'o.'

If you have specified mark guides in the main HiTRACE window (see Section 5 'Marking guides'), then the red circles will appear.

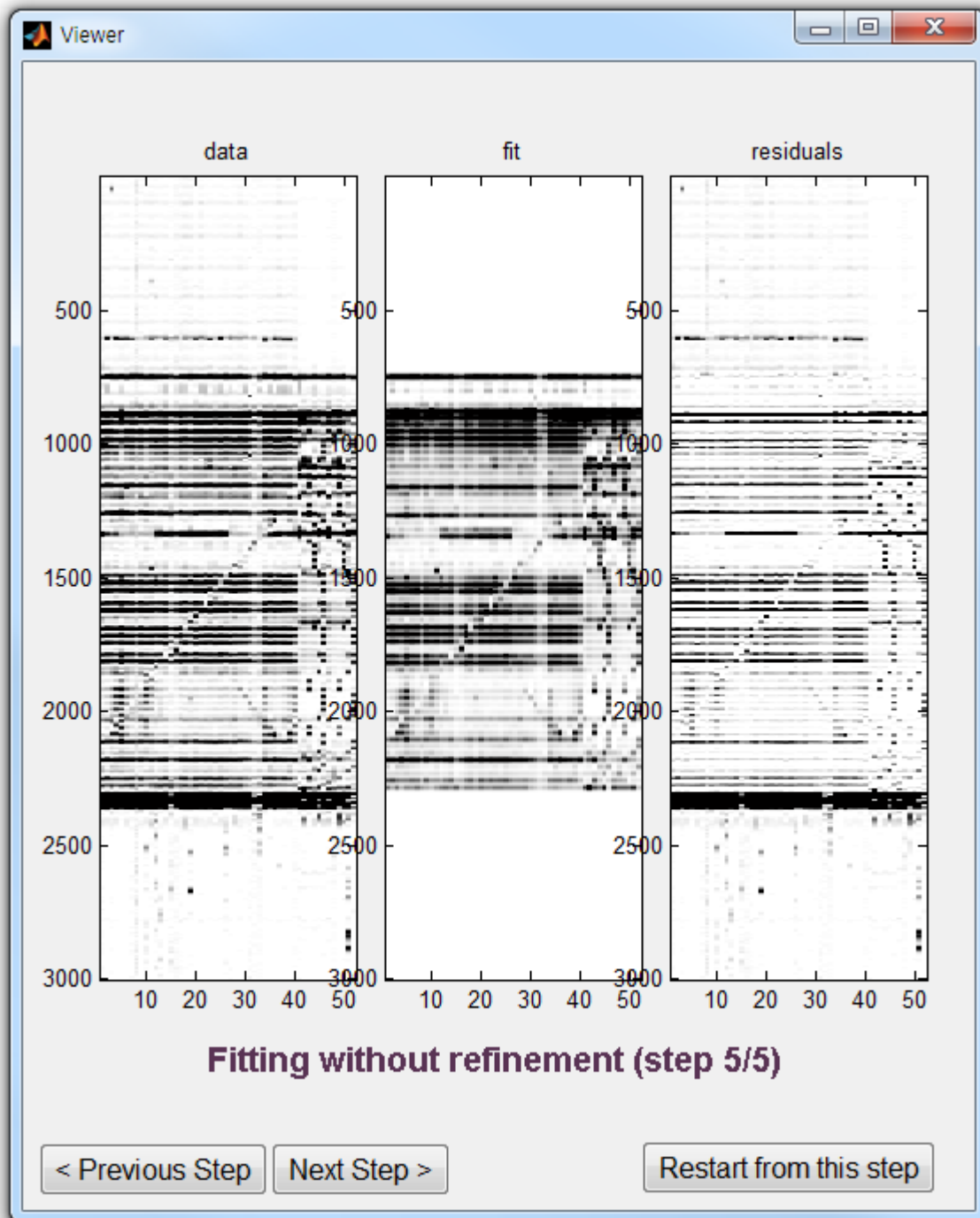
**When the manual annotation is over, make sure to click 'q' to quit from this step.**

## 10. Saving the output

In the end of an analysis run, you will see a dialog box and can save the final output by clicking 'Save data' button. The output will not be saved if 'Done' button is clicked; you can save the output later in the HiTRACE GUI window by 'Save output as text (RDAT)' button (see Section 7).



## 11. Navigating analysis steps



You can examine the result from each step by using the viewer window.

a. Previous Step

To go to an earlier step and see the result therein, click 'Previous Step' button.

b. Next Step

You can view the result from the next step by clicking 'Next Step' button in the viewer window.

c. Restart from this step

You can re-start analysis from this step by clicking 'Restart from this step' button. Before clicking this button, you can update your analysis parameters in the main HiTRACE window and clicking 'Update parameters' button (see Section 12).

## 12. Updating analysis parameters



In case you want to re-run HiTRACE analysis with new parameters, you can specify them in the main HiTRACE window and click 'Update parameters' button. Then, you can click 'Restart from this step' button in the viewer window, if you want to start your analysis from a certain step (See Section 11.c).