

Tutorial for SHAPE v.1.3

Dec. 2006
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“SHAPE” is a package of programs for evaluating biological contour shapes based on elliptic Fourier descriptors (EFDs). This package contains programs for image-processing and contour recording, derivation of EFDs, principal component analysis of EFDs, and visualization of shape variations estimated by the principal components. This tutorial gives you instructions on how to install “SHAPE” and how to try out “SHAPE” with sample files.
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! Caution - If you have been a user of the older version of SHAPE, please beware of the following point:

I found a bug in SHAPE ver.1.2, which has been fixed in SHAPE ver.1.3. Because of this bug, chain-code obtained by SHAPE ver.1.2 traces an object's contour “clockwise”, although it is expected to trace a contour “counter-clockwise” in general (you can check the direction of contour trace by the viewer programs, ChcViewer and NefViewer). EFDs derived from clockwise chain-code are different from ones derived from counter-clockwise code in the sign of coefficients b and d . As indicated in Table 1, the difference in the sign of the coefficients may not cause any serious problem in the shape analyses, if all EFD or chain-code data are obtained by the same version of SHAPE. However, it should cause a serious artifact, if you analyze jointly the data obtained by the different version of SHAPE. **Please be sure NEVER TO ANALYZE JOINTLY EFD or chain-code data obtained by THE DIFFERENT VERSION OF SHAPE!** For the joint analysis, please use [converter programs](#) to convert the direction of contour trace of chain-code or EFDs obtained by SHAPE ver.1.2.

I greatly apologize for the inconvenience.

Table 1. Direction of contour trace and problems in shape analyses

EFD and chain-code Data	Direction of contour trace	Problems in shape analyses	Ways of coping
Data obtained by SHAPE ver.1.2	Clockwise	No problem	Not necessary
Data obtained by SHAPE ver.1.3	Counter-clockwise	Of course, No problem	Not necessary
Combined data obtained by both versions	The mixture of clockwise and counter-clockwise	The difference in the direction of contour trace causes the difference in the sign of Fourier coefficients b and d . This should cause a serious artifact in the analyses	Use converter programs before analyzing the combined data

How to install "SHAPE"

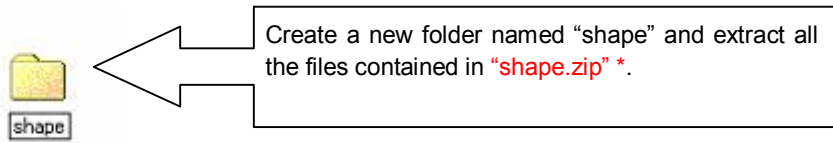


Fig.1

* In the case of the version of "SHAPE" distributed by floppy disks, extract all the files contained in "shape1.zip" (disk1) and "shape2.zip" (disk2).

1. Create a new folder named "shape" in your computer (Fig. 1).
2. Extract all the files contained in "shape.zip" to the "shape" folder.

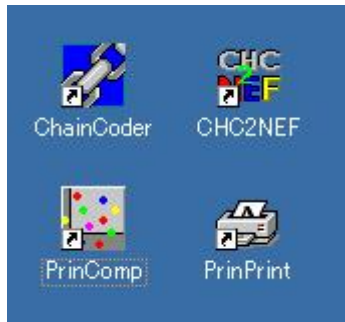


Fig. 2

(Optional) Make short cuts to "ChainCode.exe", "Chc2Nef.exe", "PrinComp.exe" and "PrinPrint.exe".

3. (Optional) For convenience, make short cuts to "ChainCode.exe", "Chc2Nef.exe", "PrinComp.exe" and "PrinPrint.exe" on your desktop (Fig. 2).

How to try out “SHAPE” with sample files

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This session gives instructions on how to try out “SHAPE” with the sample files contained in the package. First, please try out “ChainCoder” with the sample file named “Sample_img.bmp” which contains the image of five radish roots. “ChainCoder” extracts the contours of objects and stores the relevant information as chain-code. Next, please try out “Chc2Nef” with the sample file named “Sample_chc.chc” which contains chain-code data of c.a. 150 radish roots, or with the output file which you will make as a result of trying out “ChainCoder”. “Chc2Nef” provides the normalized elliptic Fourier descriptors (NEFDs) from chain-coded contours. Finally, please try out “PrinComp” and “PrinPrint” with the sample file named “Sample_nef.nef” which contains sufficient number of NEFDs data (276 radish roots) for principal component analysis. “PrinComp” performs the principal component analysis of the coefficients of the EFD, and “PrinPrint” visualizes the shape variation accounted for by each principal component. I hope that you become skilled in using “SHAPE” for your own samples through the practice of this tutorial.

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First Step: Try out “ChainCoder”

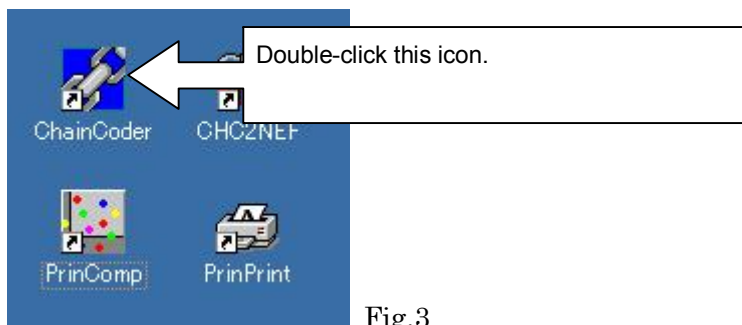


Fig.3

1. Start ChainCoder with double-clicking the “ChainCoder” icon (Fig. 3).

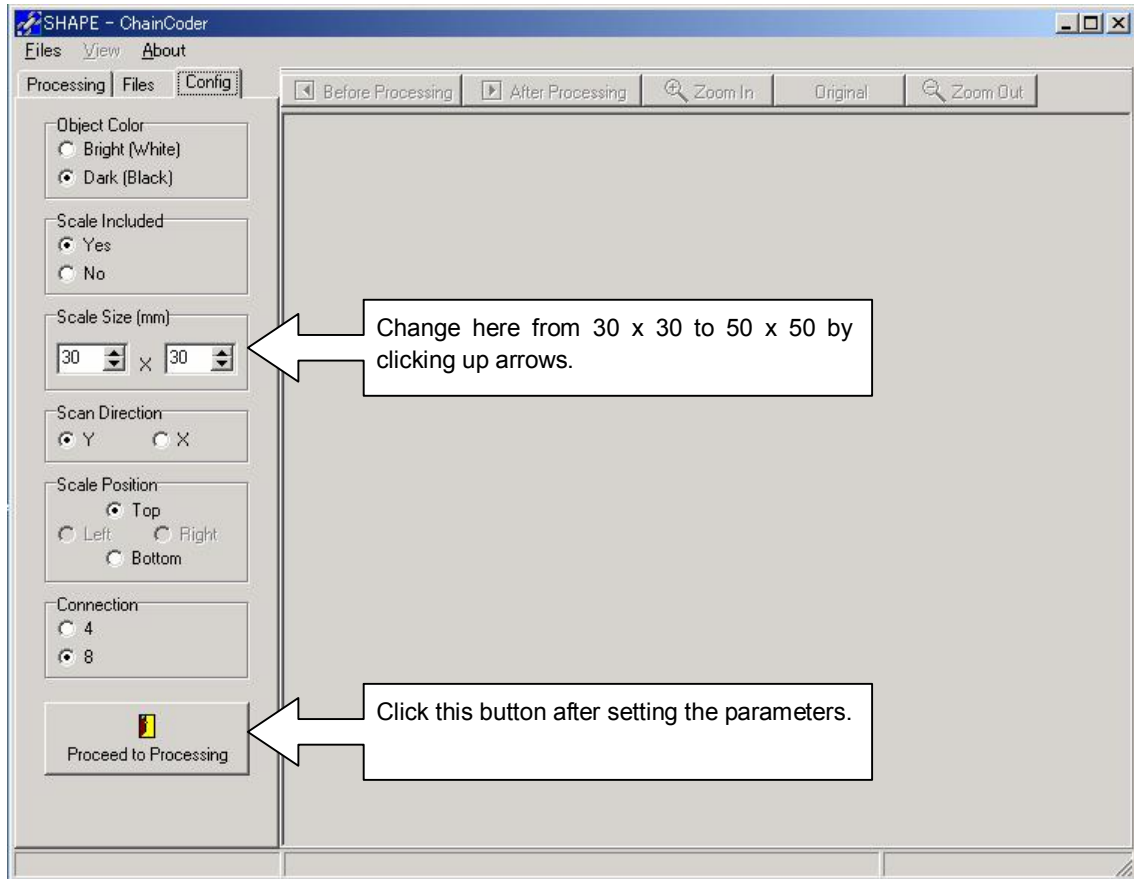


Fig. 4

2. Set or confirm the analysis parameters as follows (A-E) (Fig. 4).
 - A) Object Color -> Dark (Default)
 - B) Scale Included -> Yes (Default)
 - C) Scale Size -> 50 x 50 (mm) (Click up arrows)
 - D) Scan Direction -> Y (Default)
 - E) Scale position -> Top (Default)
3. Click the "Proceed to Processing" button (Fig. 4).

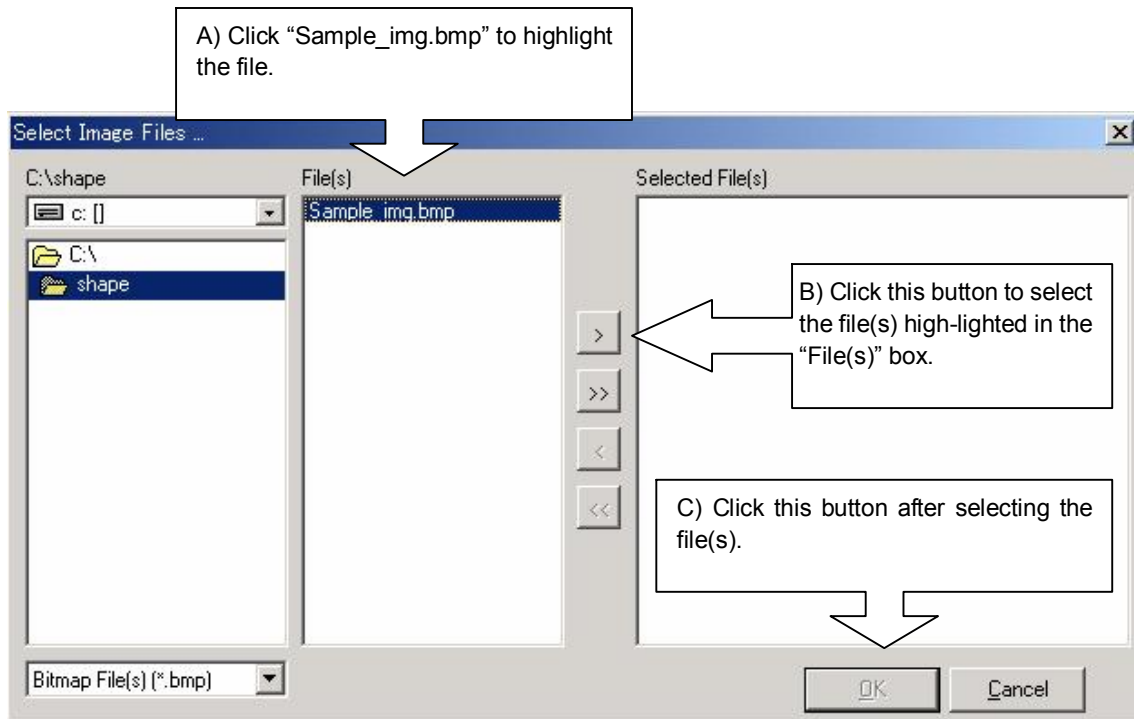


Fig. 5

4. Select the image file(s) that you are going to analyze as follows (A-C) (Fig. 5).
 - A) Click the “Sample_img.bmp” contained in the shape folder.
 - B) Click the [>] button, and “Sample_img.bmp” will appear in the "Selected File(s)" box.
 - C) Click the “OK” button.

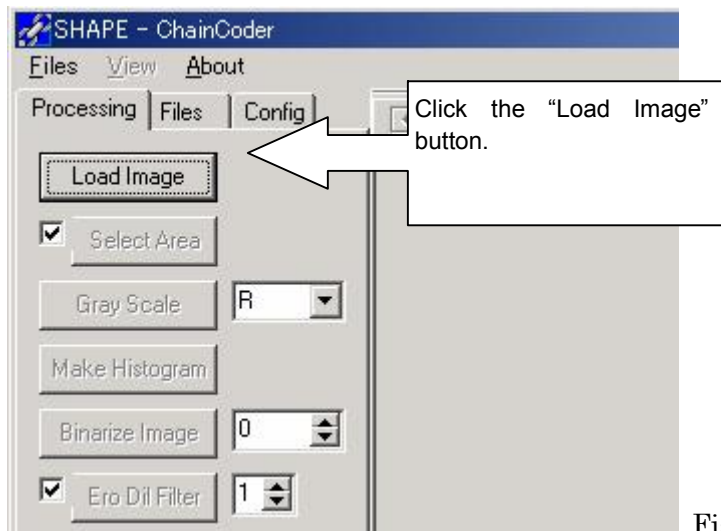


Fig. 6

5. Click the “Load Image” button to load the image into the program (Fig. 6).

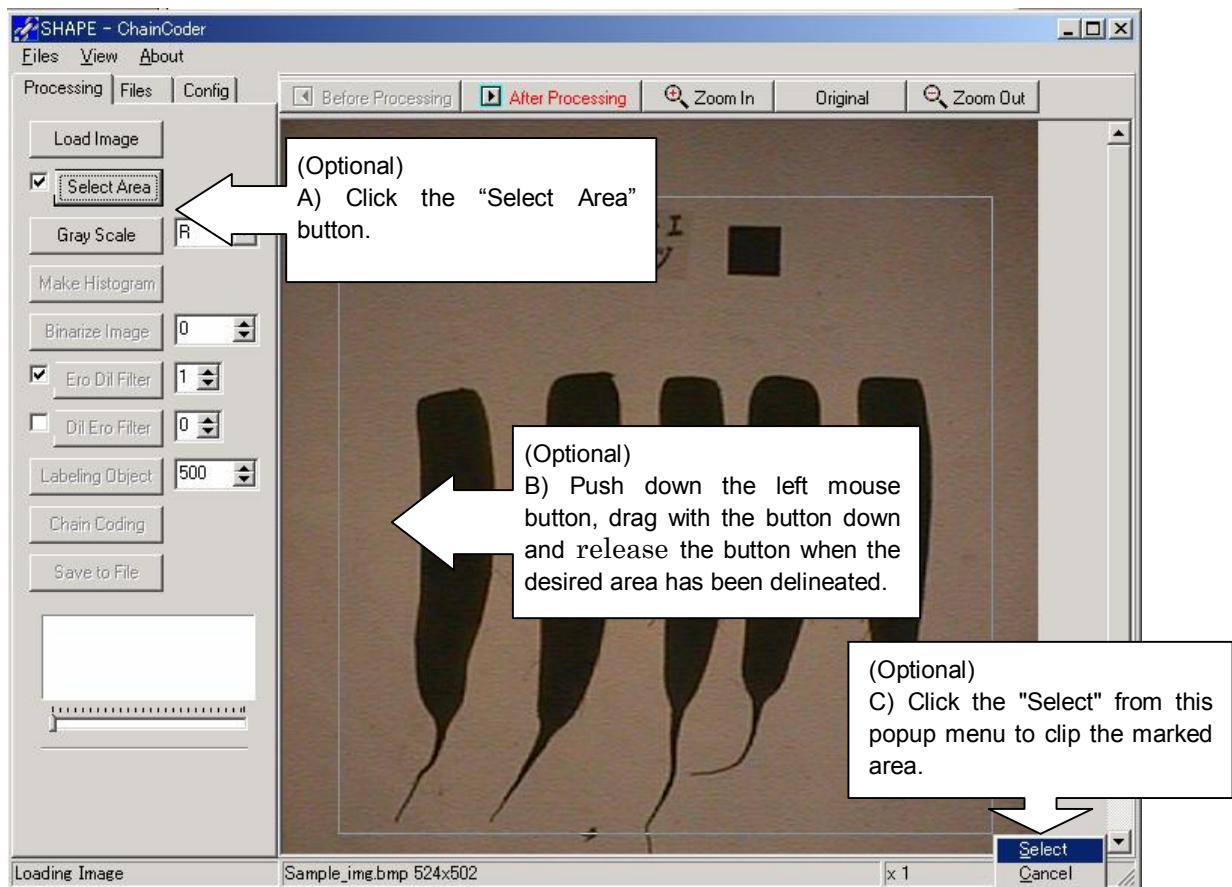


Fig. 7

6. (Optional) If you want to process the part of the image, select the processing area as follows (A-C) (Fig. 7)
 - A) Click the "Select Area" button, and the mouse cursor will change shape from an arrow to a cross in the image window.
 - B) Push down the left mouse button, drag with the button down and release the button when the desired area has been delineated.
 - C) Click the "Select" from this popup menu to clip the marked area.

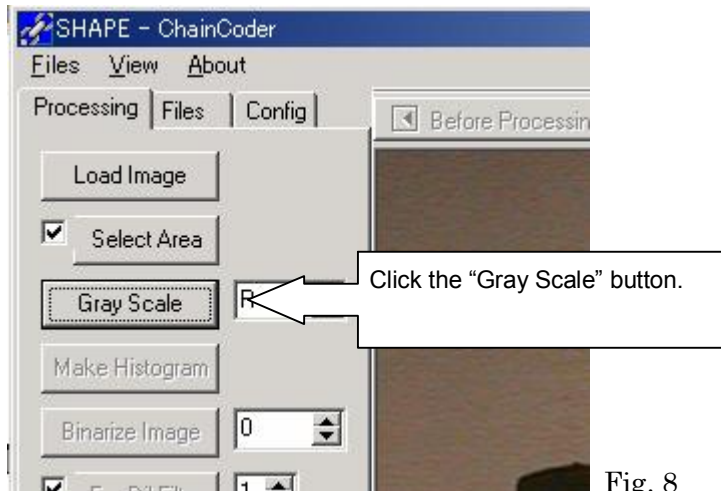


Fig. 8

7. Click the “Gray Scale” button to change a full color bitmap image to gray-scale.

! Attention: *This program can only handle FULL COLOR (24 bits) BITMAP images, and cannot handle directly 256 COLOR, 16 COLOR and MONOCHROME BITMAP images or JPEG images. So, if you have files of images in a different format, such as jpeg or less colored bitmap (black and white, 16 colors or 256 colors bitmap), you have to convert them to full color (24 bits) bitmap format prior to analysis using the graphic programs such as “Microsoft Paint” that comes with Microsoft Windows.*

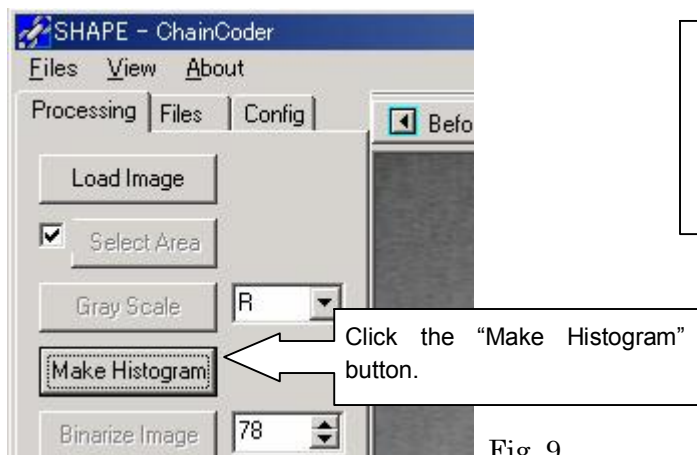


Fig. 9

A histogram of the gray scale of the pixels. In this case, the left and right peaks represent the pixels included in the objects and the background, respectively.

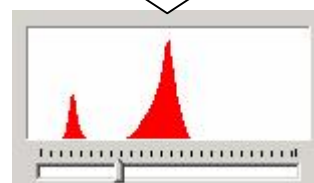


Fig. 10

8. Click the “Make Histogram” button (Fig. 9), and a histogram of the gray scale of the pixels will then be shown in the histogram box (Fig. 10). Through this step, an appropriate threshold value is determined and appeared in the box beside the “Binarize Image” button.

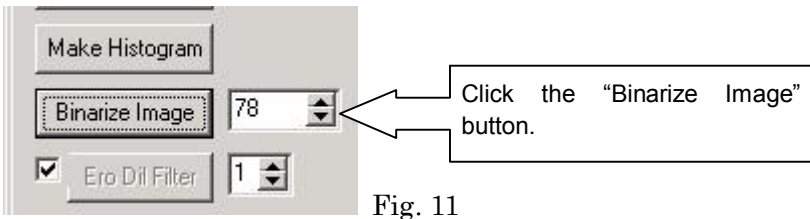


Fig. 11

- Click the "Binarize Image" button, to convert the gray scale image to a binary image in which the objects and background are represented as 1 (white) and 0 (black), respectively (Fig. 11). If you want to adjust threshold value for the binarization, change the value in the box beside the "Binarize Image" button by clicking the up or down buttons, as appropriate (Fig. 11). The value can be changed by dragging the pointer on the ruler bar below the histogram box (Fig. 10).

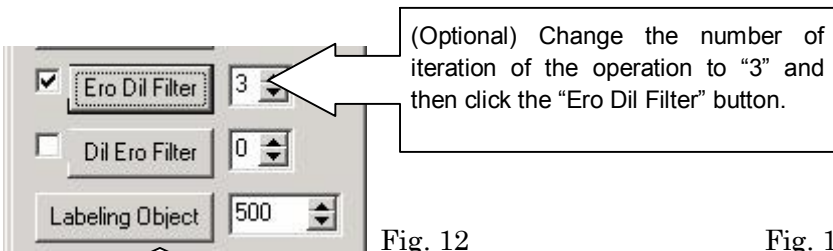


Fig. 12



Fig. 13

Click the "Labeling Object" button.

The thin part of the root is removed after the operation.

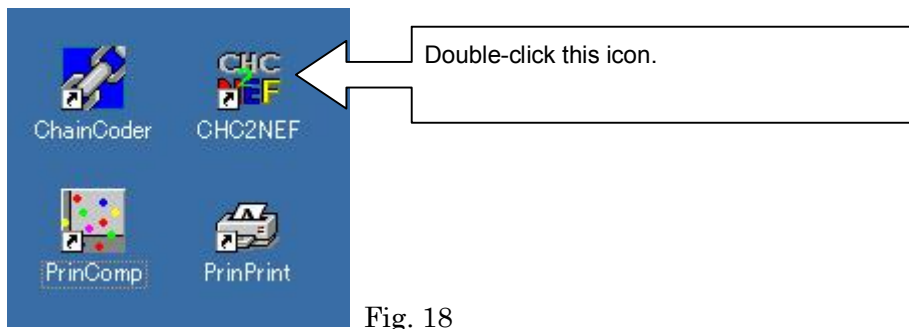
- (Optional) Change the number of iteration of the operation from "1" to "3" and click the "Ero Dil Filter" button (Fig. 12). Then, the noises and the thin part of the roots will be removed (Fig. 13).

Chain Code Data					
Name	Sample_img	Scale No.	1	Area/Pixels	2.196837E+00
Data Name	No.	SX	SY	Area	Chain Code
1	JSCALE1	297	70	1138	
2	Sample_img	1	203	167	10259
3	Sample_img	2	258	170	8139
4	Sample_img	3	320	170	8863
5	Sample_img	4	390	170	8397
6	Sample_img	5	117	178	9256

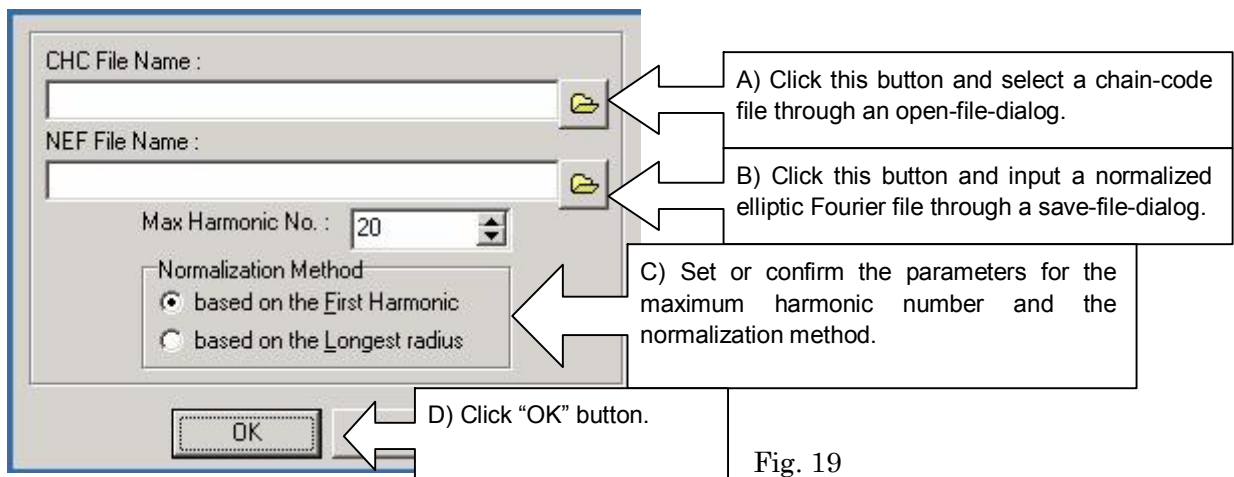
Fig. 14

- Click the "Labeling Object" button (Fig. 12), and each object will then be numbered and displayed in the "Chain Code Data" window (Fig. 14).

Second Step: Try out “Chc2Nef”



15. Start “Chc2Nef” with double clicking the “Chc2Nef” icon (Fig. 18).



16. Set analysis parameters as follows (A-D) (Fig. 19).

- A) Click the button beside the “CHC File Name” box and select a chain-code file through an open file dialog. In this tutorial, please select “Sample_chc.chc” or “test.chc” which will be obtained on the step 13 in this tutorial.
- B) Click the button beside the “NEF File Name” box and input a normalized elliptic Fourier file through an save file dialog. If you select “Sample_chc.chc”, the default name of the save file is set to “Sample_chc.nef”.
- C) Set “Max Harmonic No.” as “20” and “Normalize Method” as “based on the First Harmonic”. For detail information for these parameters, see the manual.of SHAPE v.1.3.
- D) Click “OK” button.

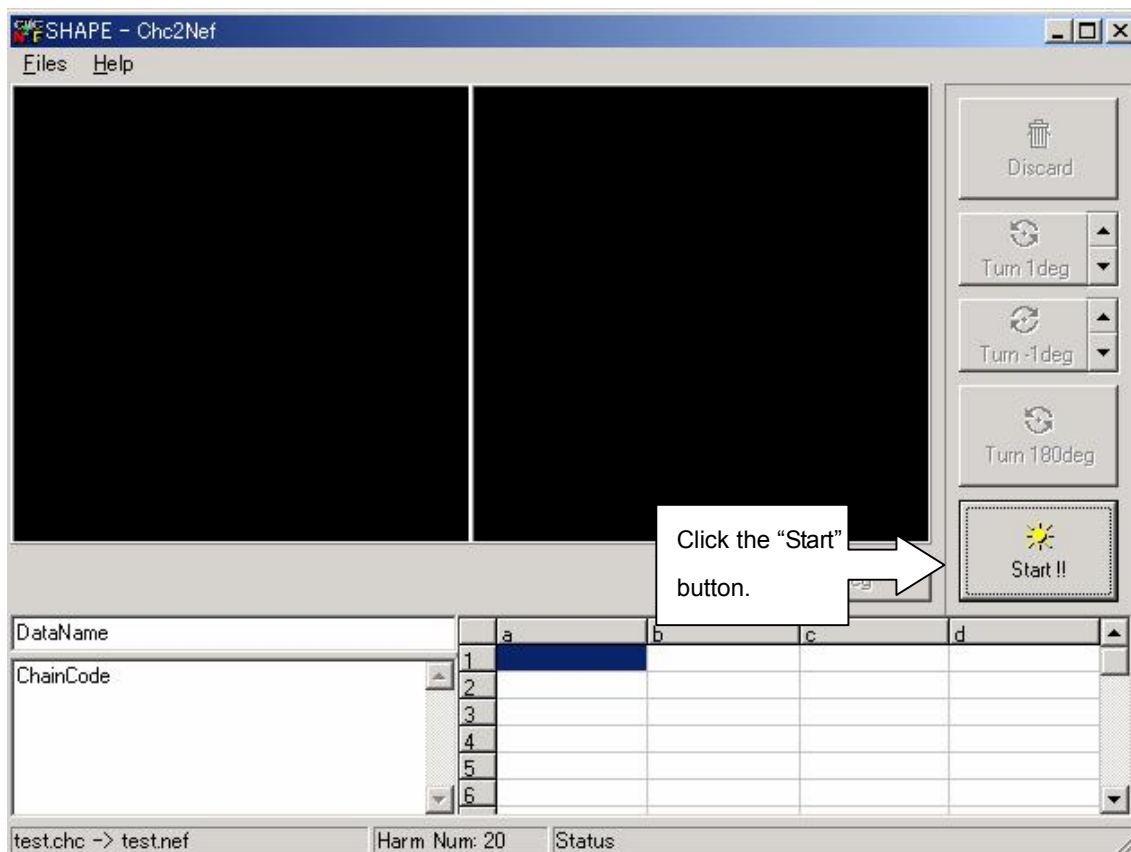


Fig. 20

17. Click the "Start" button (Fig. 20). The chain code of the first object is then converted to the normalized elliptic Fourier descriptors (NEFDs) and the NEFDs and the contour reconstructed by the NEFDs will be appeared in the window (Fig. 21).

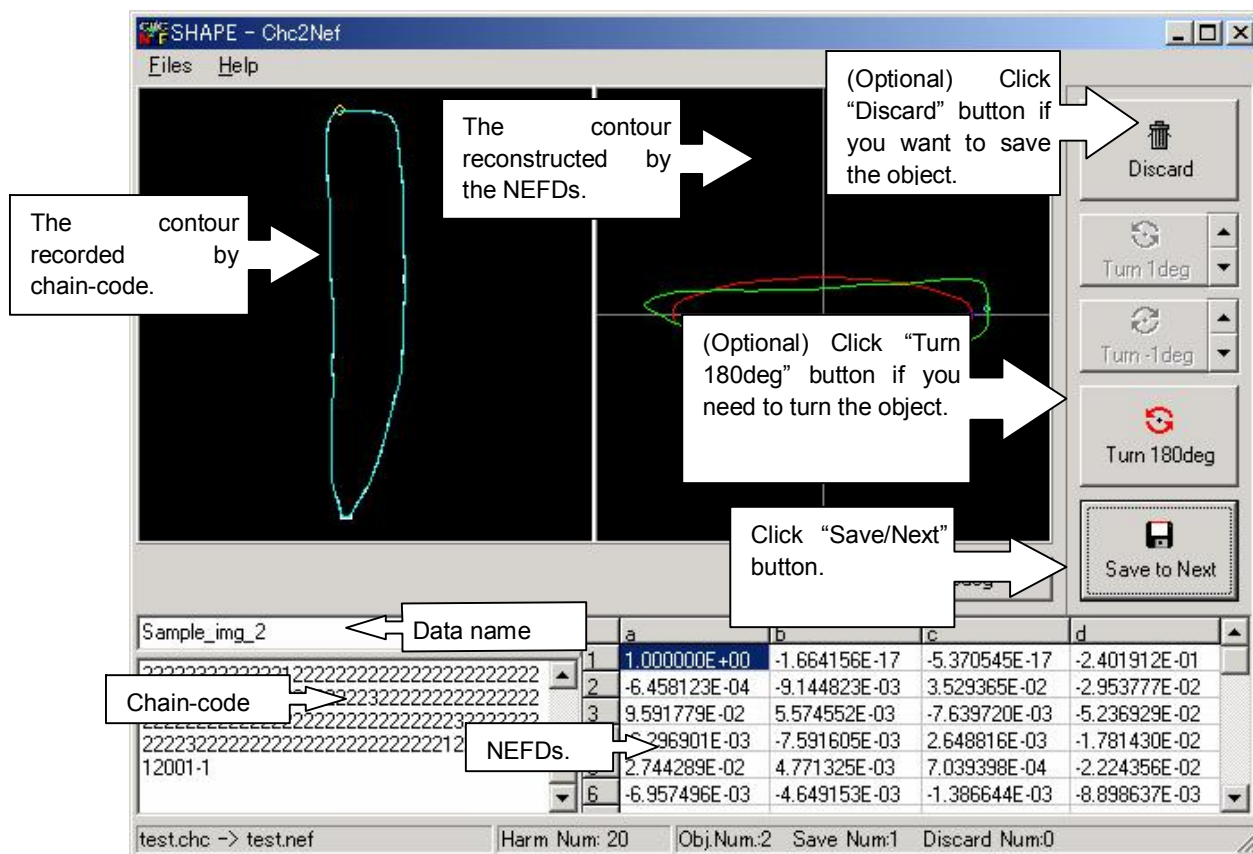


Fig. 21

18. (Optional) Click “Turn 180deg” button if you need to turn the object (Fig. 21).

19. (Optional) If you don't want to save the object, click the "Discard" button (Fig. 21).

20. Click “Save/Next” button. Then, the NEFDs obtained will be saved to the output file and the chain-code of the next object will be converted to normalized elliptic Fourier descriptors (Fig. 21). To convert the remaining objects, repeat the steps 18 and 19 until all the objects have been converted.

21. Close “Chc2Nef”.

Final Step: Try out “PrinComp” and “PrinPrint”

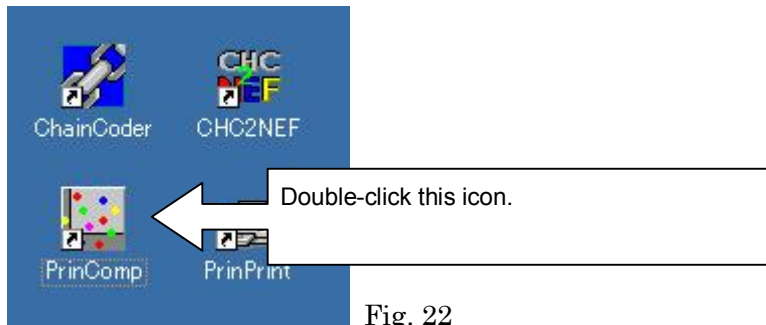


Fig. 22

22. Start “PrinComp” with double clicking the “PrinComp” icon (Fig. 22).

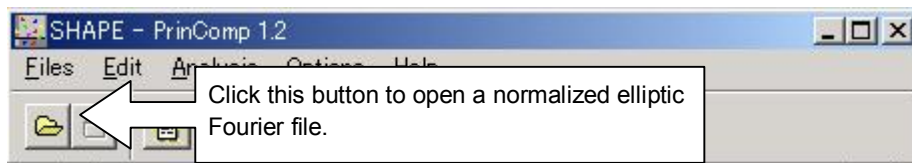


Fig. 23

23. Click “open-file” button (Fig. 23). An open-file-dialog will appear. Then select the sample file named “Sample-nef.nef”. (You can also select the normalized elliptic Fourier file obtained through this tutorial. In that case, the result of principal component analysis will, however, not be meaningful because the number of samples will be small for principal component analysis.)

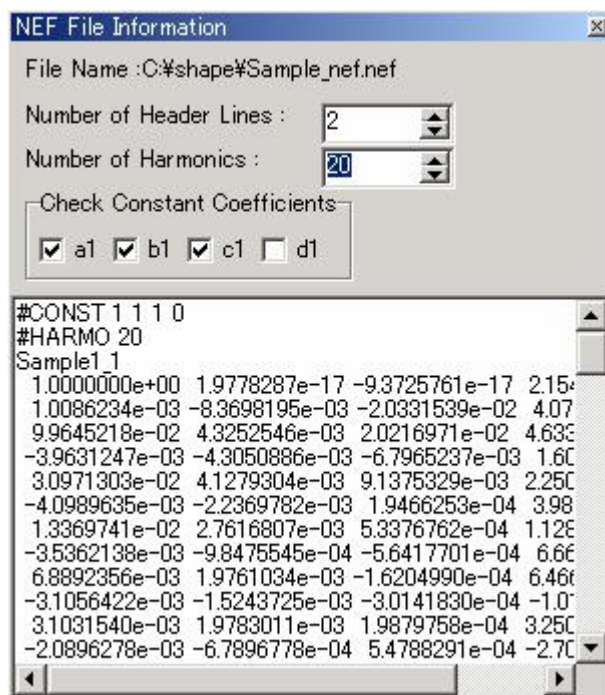
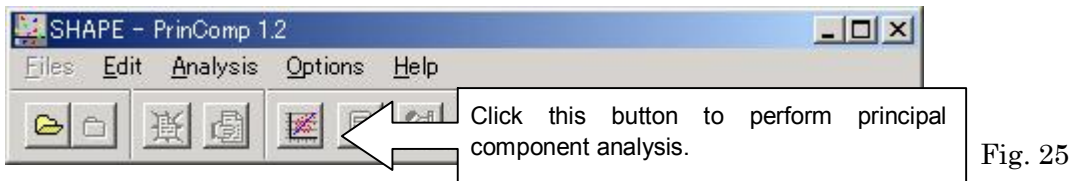
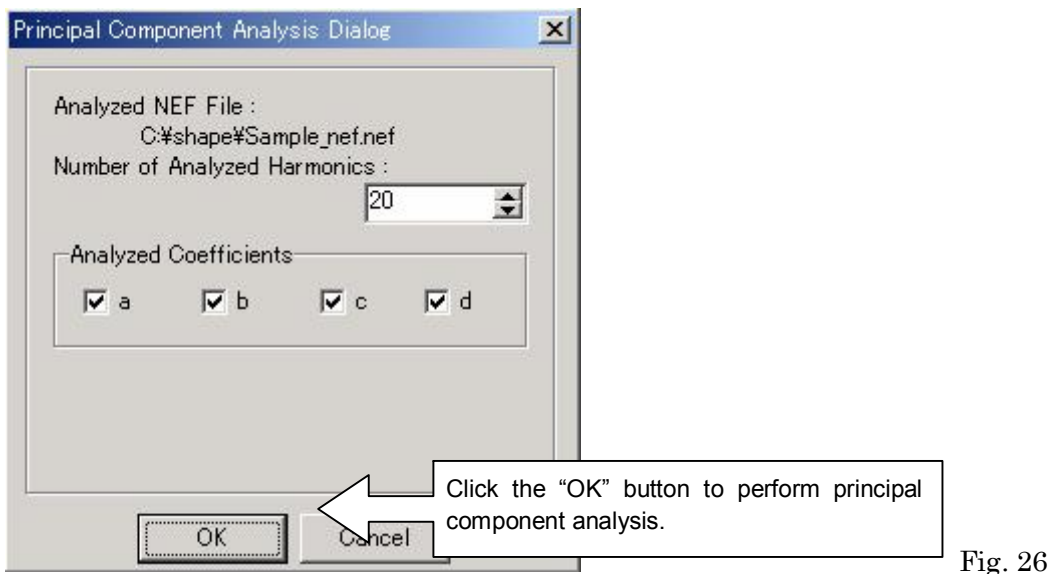


Fig. 24

24. After selecting the normalized elliptic Fourier (NEF) file, The "Nef File Information" window will appear. The "Number of Header Lines", "Number of Harmonics" and "Constant Coefficient" parameters are automatically set according to the information described in the header in the NEF file (Fig. 24).



25. Click the analysis button to perform principal component analysis (Fig. 25) and "Principal Component Analysis Dialog" (Fig. 26) will be appeared.



26. Click "OK" button (Fig. 26), and the save file dialog will be appeared. After specifying the name of a saved file (*.pcr), principal component analysis will be performed and the information window which contains the result of principal component analysis will be appeared (Fig.27).

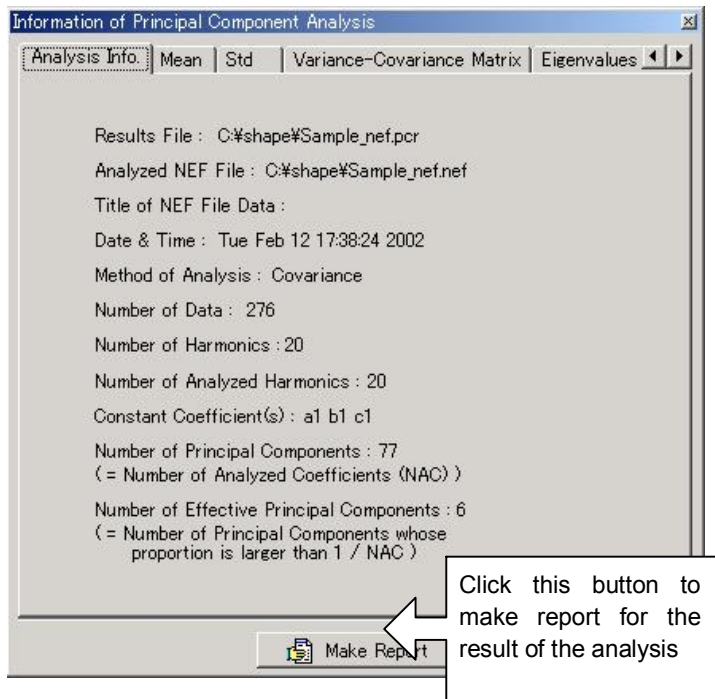


Fig. 27

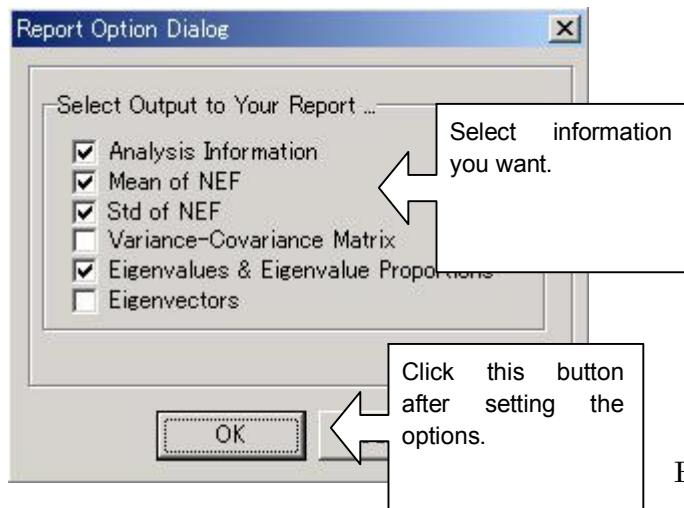


Fig. 28

27. Information contained in “Information of Principal Component Analysis” window can be output to a result report file in text format. Click “Make Report” button to make a result report (Fig. 27), and the “Report Option Dialog” will be appeared (Fig. 28). Set the options for the result report, and click the “OK” button. Then, the save dialog will be appeared. Input a file name to the dialog and click the "Save" button, a result will be saved in a report file.

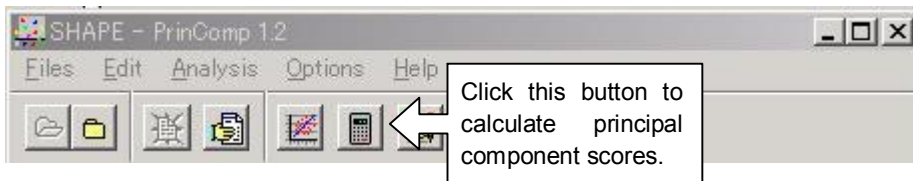


Fig. 29

28. To calculate the scores of principal components, select "Calculate Prin Score" button (Fig. 29). Then, the "Prin Score Dialog" will appear.

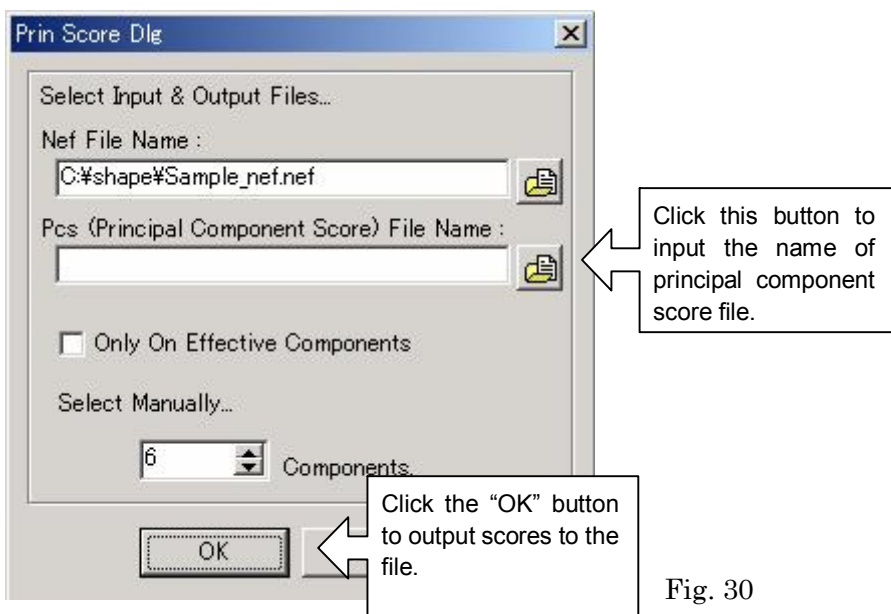


Fig. 30

29. Select the NEFDs file (which is automatically selected by the program) and input the principal component score file name (*.pcs) (this is done automatically by clicking the button beside the box) (Fig. 30). You can also change the number of the components for which the scores are calculated. Then, click the "OK" button. The file will then be saved in tabbed text format and can be opened as a Microsoft Excel worksheet for succeeding analysis from the appropriate (e.g. biological) perspective.



Fig. 31

30. To visualize the shape variation explained by each principal component, click the button with a line-drawing graphic (Fig. 31). After that, the "Reconstruct Contours Dialog" will be appeared.

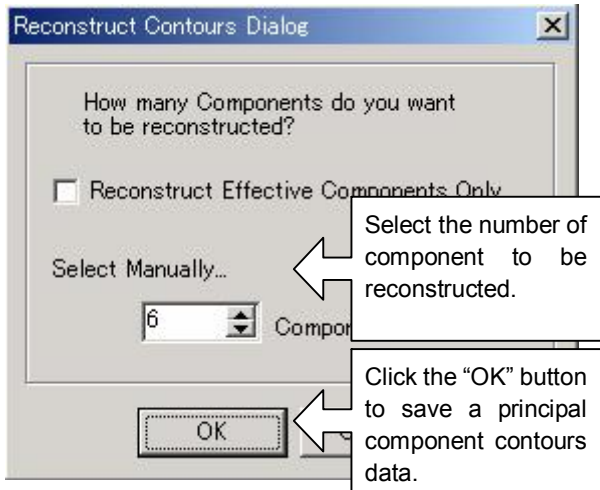


Fig. 32

31. Select the number of components to be reconstructed through the "Reconstruct Contours Dialog" (Fig. 32). Click the "OK" button, and a save dialog will appear. Input the name of a principal component contours file and click "Save". After that, "PrinPrint" will be automatically executed to display the result and the contours are displayed in a preview form for printing (Fig. 33). If "PrinPrint" cannot be automatically executed, start "PrinPrint" with double clicking the "PrinComp" icon and open the principal component contours file by selecting "Open" from "Files" menu.

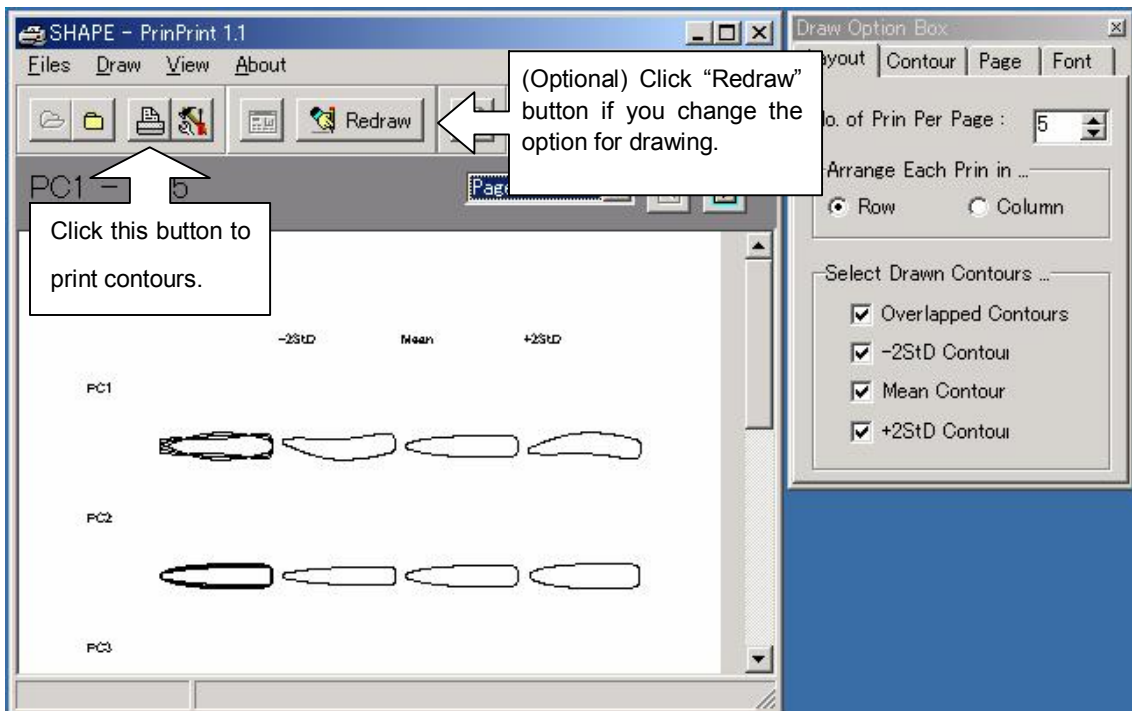
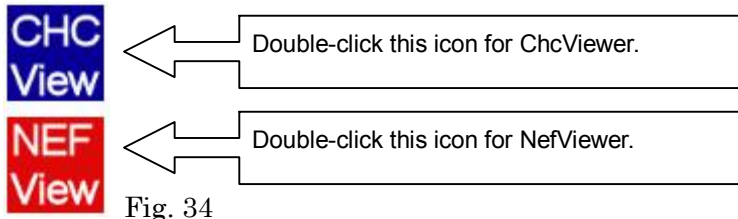


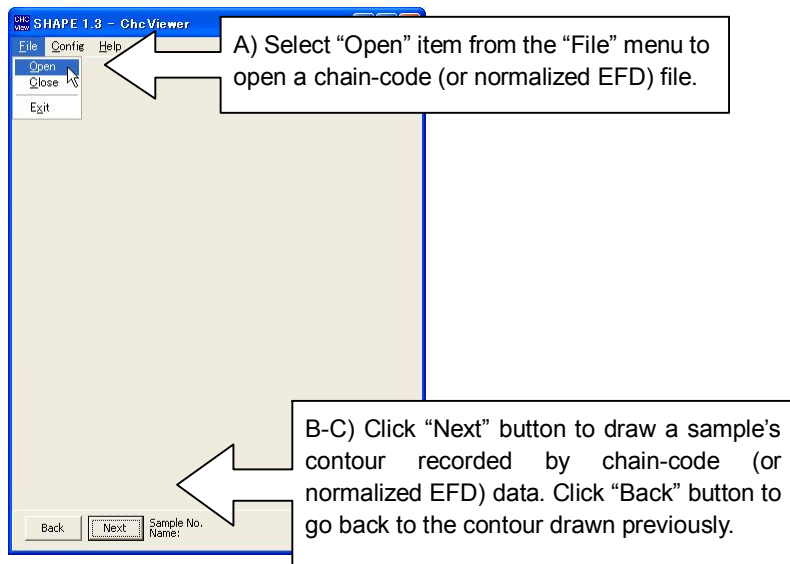
Fig 33

32. (Optional) You can change some options for drawing the contours, if desired. After setting the draw options, click the "Redraw" button (Fig. 33), the preview window will then be updated in accordance with the new settings.
33. Click the button with a printer icon (Fig. 33), and the print dialog will appear. After setting the printer properties, click the "OK" button. You can then print the contours.
34. Close "PrinComp" and "PrinPrint".

Optional Step: Try out “ChcViwer” and “NefViwer”



35. Start “ChcViewer” (or “NefViewer”) by double-clicking the “ChcViewer” (or “NefViewer”) icon (Fig. 18).



36. View the sample’s contour recorded by chain-code (or normalized EFD) data as follows (A-C) (Fig. 35).

- A) Select “Open” from the “File” menu, and select a chain-code (or normalized EFD) file through an open file dialog. In this tutorial, please select “Sample_chc.chc” (or “sample_nef.nef”).
- B) Click “Next” button. The contour of the first sample will appear in the window.
- C) Click “Next” button to draw the contour of the next sample. Click “Back” button to go back to the previous sample.

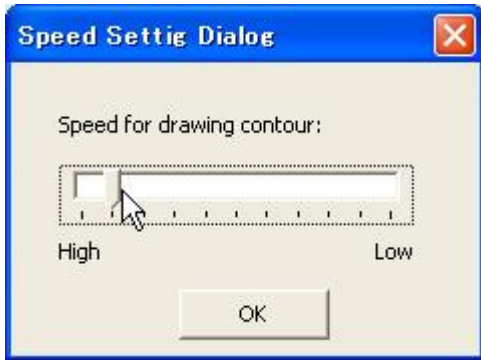


Fig. 36

37. (Optional) You can change speed for drawing a contour. Select "Speed" from the "Config" menu, the "Speed Setting Dialog" window will appear (Fig. 36). The speed can be changed by dragging the pointer on the ruler bar in the "Speed Setting Dialog" window.

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Through this tutorial, you may become familiar with the process of elliptic Fourier analysis performed by “SHAPE”. Next, please try out “SHAPE” with your own samples. I hope that SHAPE will be used by many researchers in diverse fields and that it will help elucidate various important aspects of biological shapes in the future.
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