NeuRA

The Neuron Reconstruction Algorithm

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1 Introduction

1.1 What is NeuRA?

NeuRA is a program for automatic convertion of image stacks, which are aquired by 2-photon microscopy, into vector diagrams.
[...]

1.2 About this manual

As can be seen in the table of contents, we first give an general overview of NeuRA and then have a closer look at the specific parts of the program.
It is useful to study the figures, too, because they often give additional descriptions.
In fact, it should be possible to make a first test of NeuRA, just by follow the instructions in the yellow bubbles.

TIP: If there is a problem, look for these yellow marked tips. Often they give useful field-tested hints.

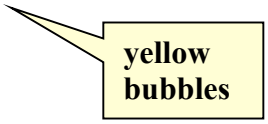
1.3 Manual Conventions

1.3.1 Used abbreviations

LMC	: =	left mouse click
DLMC	: =	double left mouse click
<ctrl>	: =	press control key on the keyboard
<shift>	: =	press shift key on the keyboard
<ret>	: =	press return key on the keyboard
<tab>	: =	press tabulator key on the keyboard
<rArr>	: =	press the right arrow key
<lArr>	: =	press the left arrow key

1.3.2 Text markers

Button labels are *Italicized And in Upper Case Writing*.
Possible file types begin with a dot (.) and are *italicized*, too.
Important texts are marked yellow.
Keys are in <angle brackets>.
Other abbreviations are in UPPER CASE LETTERS.
Instructions for the figures are in .



2 Installation of NeuRA

3 Working with NeuRA

(see fig.1)

3.1 Starting NeuRA

You can start NeuRA
with **LINUX**: by typing the command `neura` in a terminal,
with **Windows**: by DLMC on the NeuRA symbol on the screen,
with **MAC**: by LMC on the NeuRA symbol on the screen.
A screen like in fig. 1 will appear.

3.2 Layout

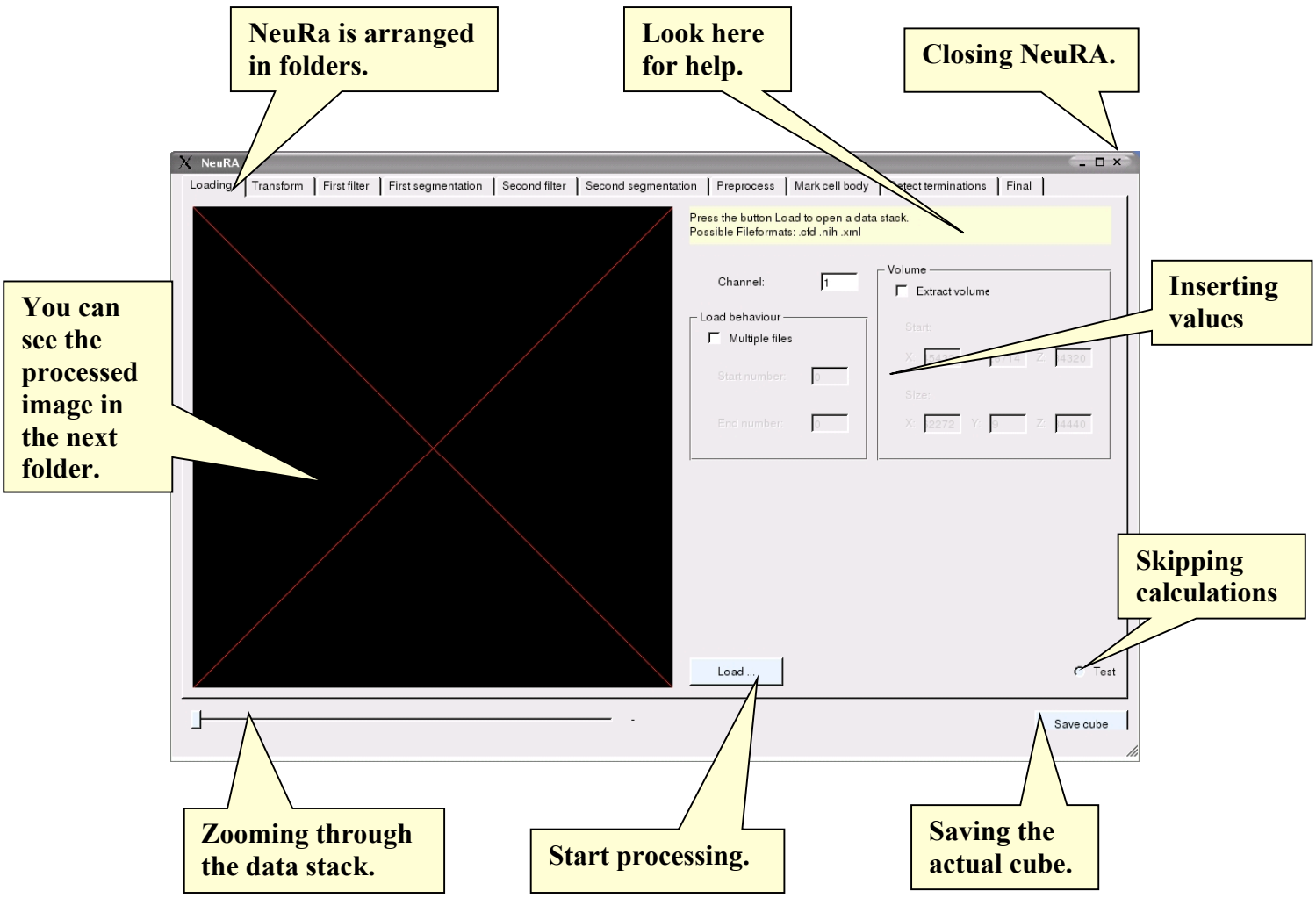


figure 1: Layout

3.3 Help

Each folder provides a yellow help box in the upper right corner, which contains information about how to proceed.

3.4 Image Screen

If there is no image loaded resp. processed yet, the black image screen will show a red cross. You can see the processed image on the image screen of the next folder; excluding the loaded image, which is shown in the first and in the next folder.
Zoom through the image stack by drawing the button over the bar below the image screen with the mouse (keep LMC pressed while drawing). Alternatively you can mark the bar by repeatedly pressing <tab> until the bar is marked, and then use < lArr> and <rArr> to zoom.
The number of the image will be shown at the right side of the bar.

3.5 Settings

On the right of the image screen are boxes in which you can insert values.
If you are not sure work with the defaults.

TIP: Look for hints in the help box.

3.6 Processsing the image

If every value is set, just press the button on the bottom right side of the image screen. NeuRA will compute the results and show them in the next folder.

3.7 Skipping calculations

You can also load image stacks, which are already processed. Then you can skip the concerned calculations by marking the *Skip Calculation* button in the bottom right corner with a mouse click.

TIP: This is a very useful feature of NeuRA, if you have to break your work. You can save the actual cube and load the processed one later, skipping all calculations until you get to the break point.

3.8 Saving of datacubes

You can save the actual datacube by pressing the *Save Cube* button on the folder where the processed image is shown. (Remember: the processed image is in the next folder.)

TIP: Save at least after filtering and of course after the final reconstruction.

3.9 Closing NeuRA

Close NeuRA by pressing the close button (e.g. *X*) on the upper right corner of the folder.

4 Folders

4.1 Loading

(see fig.2 and fig. 3)

To open an image stack press the *Load* button (see fig. 2) and choose a file from the pop-up menu .(see fig.3)

Possible file formats are: *.cfd*, *.xml*, *.nih*, *.tiff*.

Channel: [change to 2]

Load behaviour: [not yet implemented]

Multiple files: To save time, it is possible to load several files at the same time.
Start number: number of first file to load.
End number: number of last file to load.
All files between (and including) these will be loaded and processed in succession.

Volume:
Extract volume: It is possible to load only a part of the image stack.
Start: identifies the bottom left front corner.
Size: describes the size of the partial image in all three space dimensions (x, y, z) and is measured in voxel number.

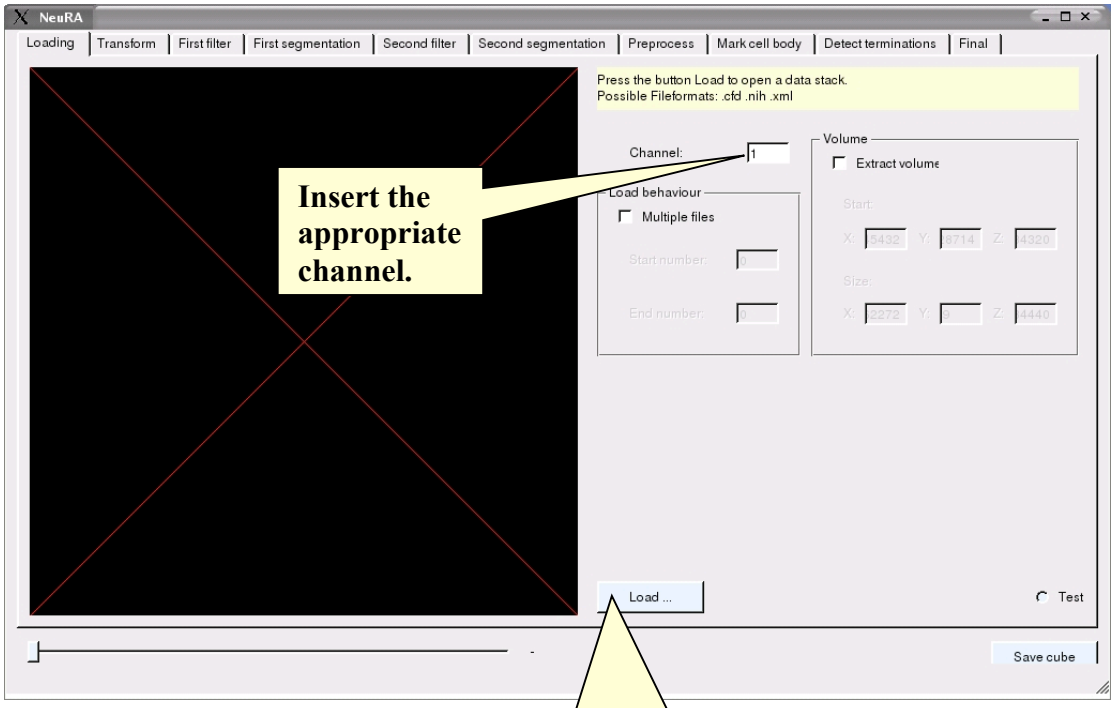


figure 2: Loading

Press this button to load a file. A pop-up menu will appear. (see fig. 3)

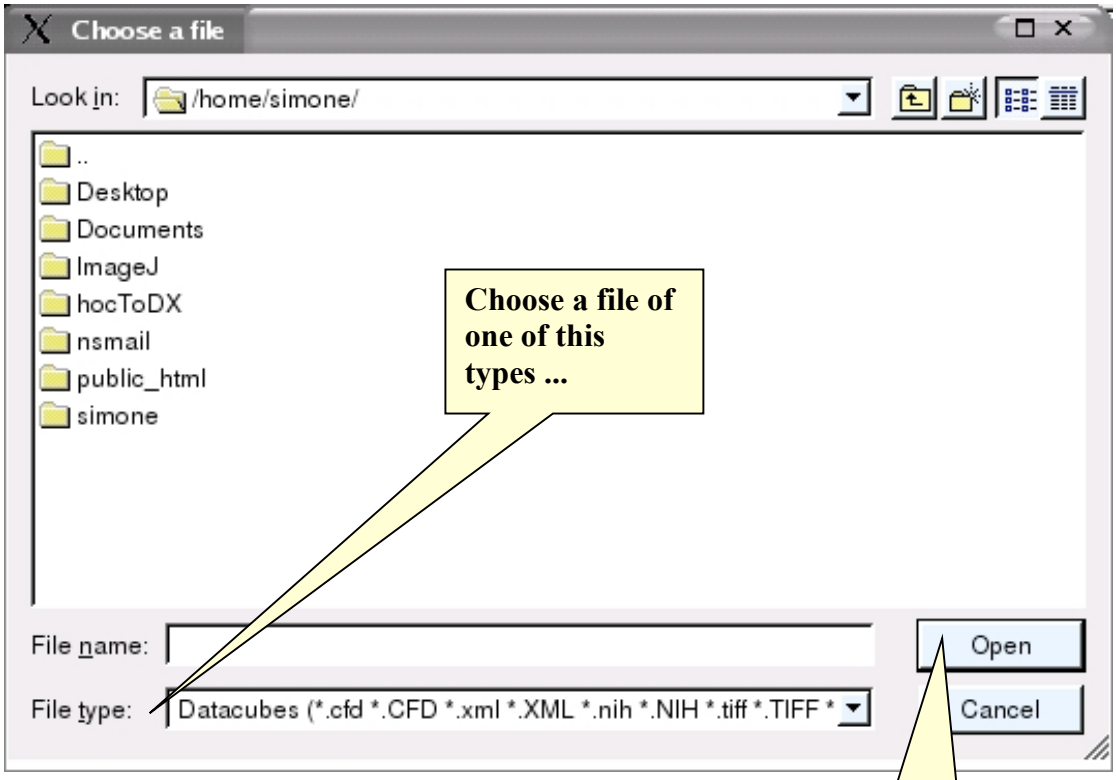


figure 3: Choose a file

... and press this button.

TIP: You can also load preprocessed files. Just skip the already performed calculations.

4.2 Transform

(see fig.5)

Transforming effects a contrast amplification.

Threshold low: All gray values below this value will be set to 0.
It is reasonable to set this value to 0.

Threshold high: All gray values over this value will be set to 1.

All values between these two thresholds will be interpolated. [see figure 4 below]

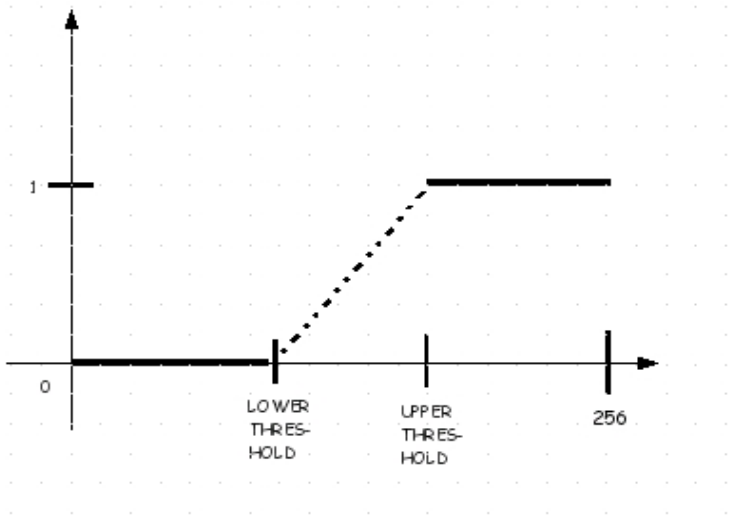


figure 4: Example function for transforming

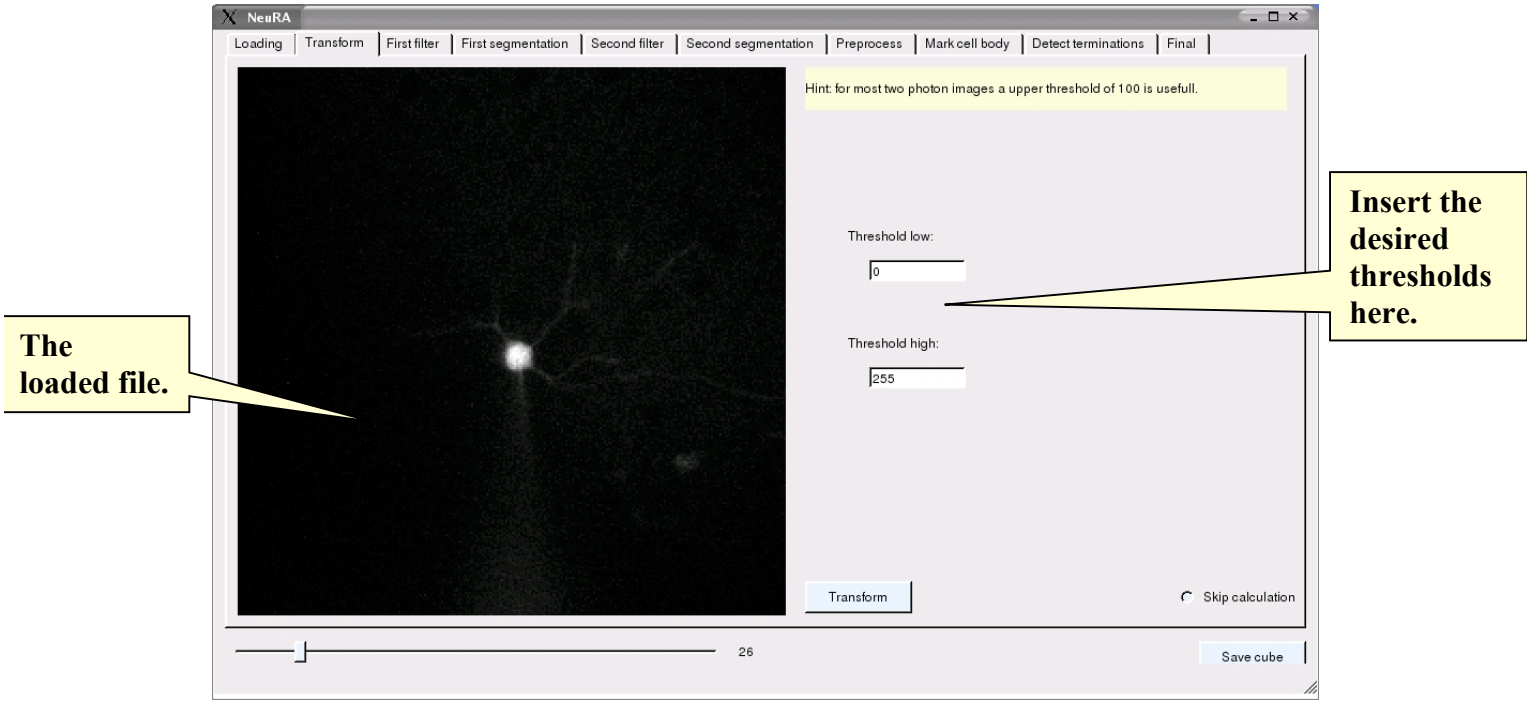


figure 5: Transform

TIP: For most to photon images an upper threshold of 100 is useful.

4.3 First filter

(see fig. 6)

The filtering removes the noise originated from the recording. The micrographed structure is preserved resp. amplified.
If it is possible, discontinuities of the structure will be filled.

Solver: [...]

Tau: [...]
Epsilon: [...]
[-> link to philip's homepage]
Time steps: [...]
Levels: [...]
Solver type: [...] CG Solver, Multigrid

Filter level:[...]

Discretization: [...]

Diffusion: [...] Nonlinear, Linear
Non-linear type: [...] Perona Malik, Weikert, Black Sapiro
Kind of moments: [...] Nodewise, Elementwise

Integration point: [...] Usual, Boundary
Aniso coefficients: [...]
#1: [...]
#2: [...]
#3: [...]
Lambda: [...]
Integration size: [...]

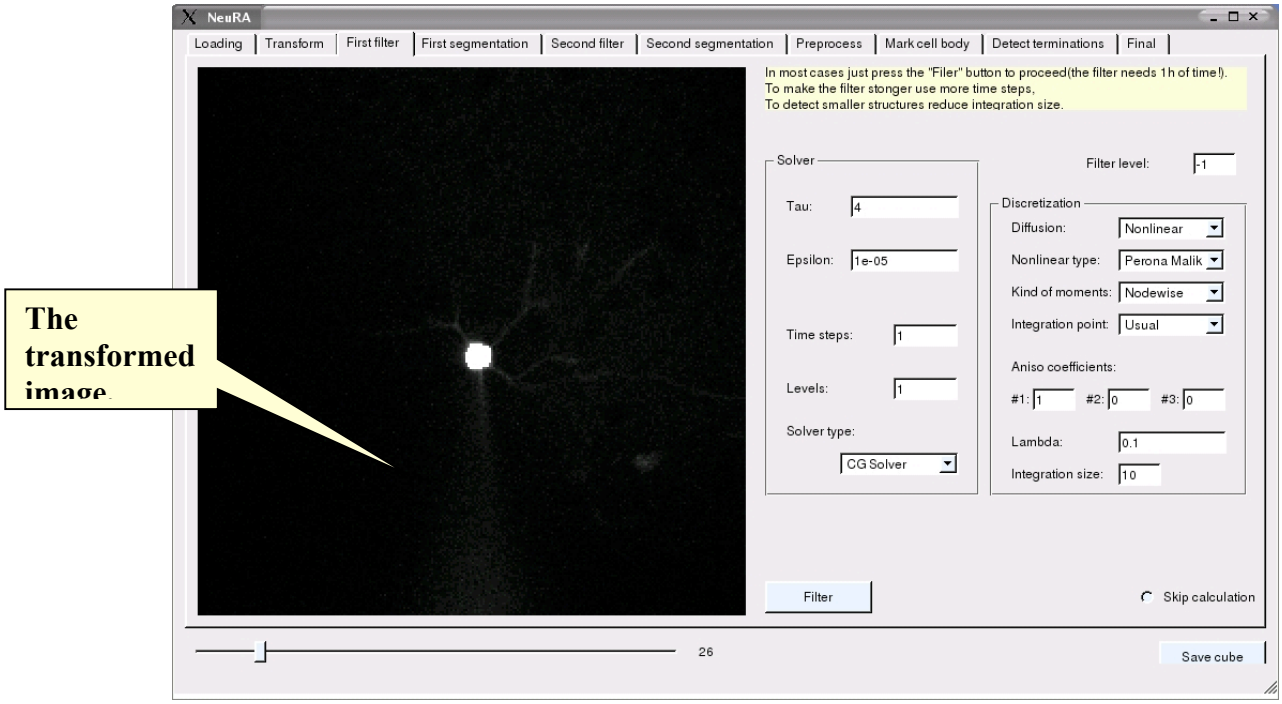


figure 6: First filter

TIP: In the most cases just press the *Filter* button to proceed. The filter will need about one hour. To make the filter stronger use more time steps. Reduce the integration size to detect smaller structures.

4.4 First Segmentation

(see fig. 8)

Segmentation means seperating the image into structure and background.

Before segmentation it is useful to buffer the filtered datacube.
(see fig. 7)
You can save the image as: *.raw*, *.vrd*, *.xml*, *.nih*, *.tiff*, *.tif*

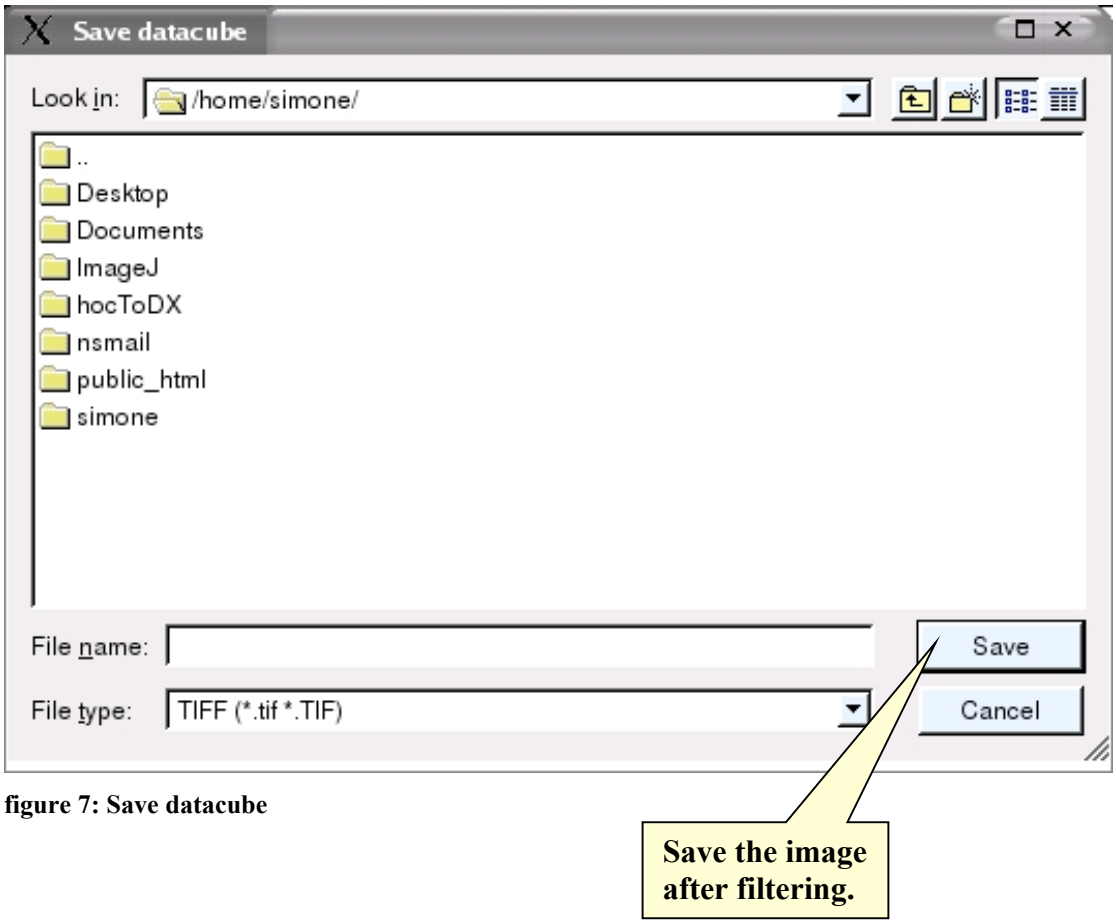


figure 7: Save datacube

Segmentation type: [...] [choose local mean values and neighbours]

Global Thresholding
Local mean values and neighbours -> figure[]
Otsu
Fast marching {not yet implemented }

Epsilon: [...]

Absolute low threshold: [...]

Absolute high threshold: [...]

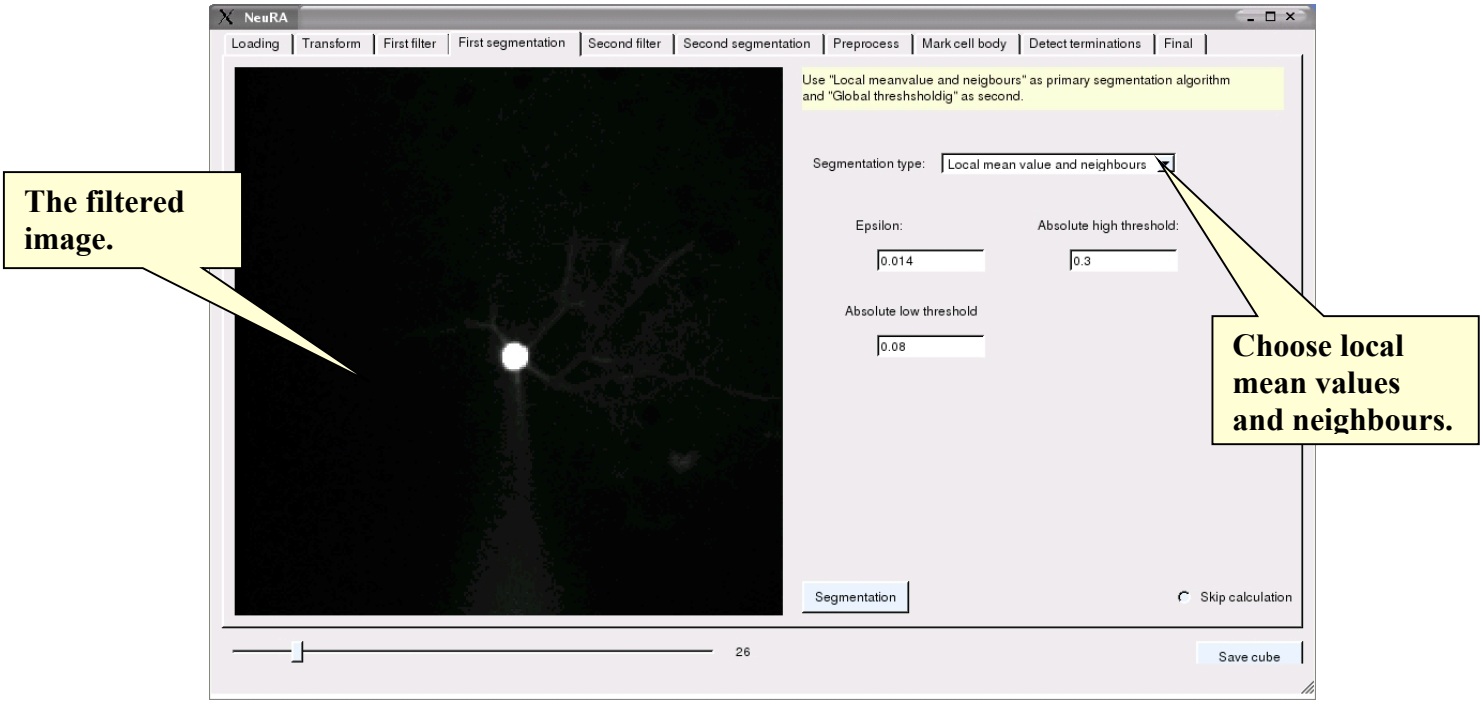


figure 8: First segmentation

4.5 Second filter

[like first filter]

(see fig.9)

A second filtering is favorable for a good reconstruction.

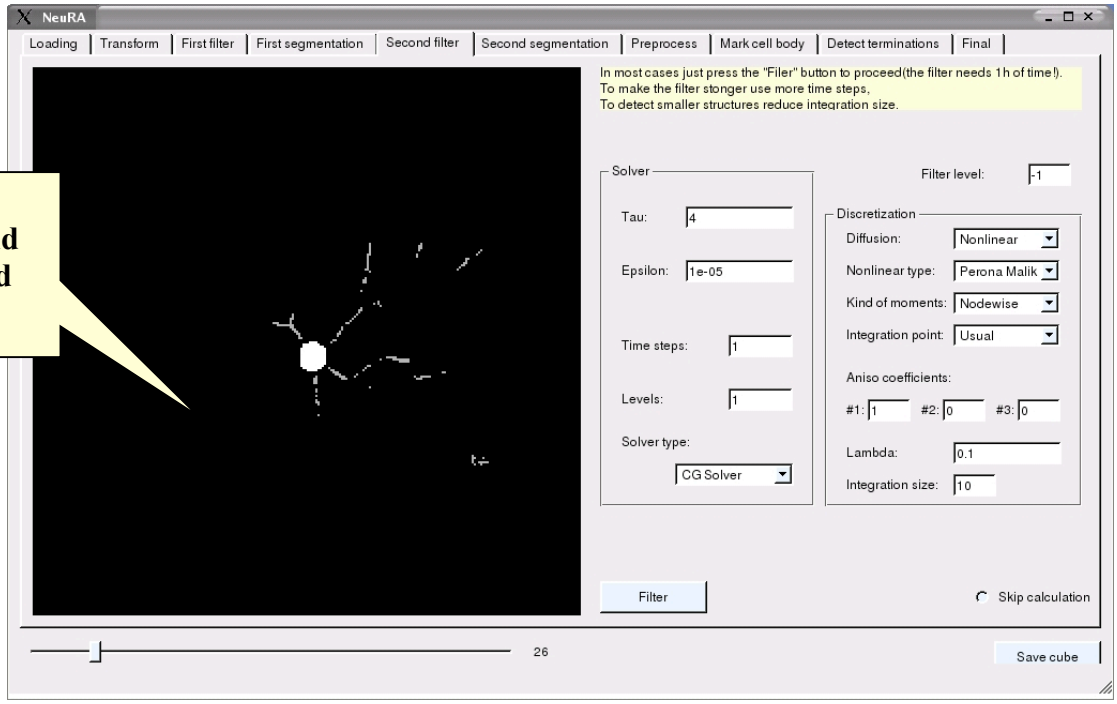


figure 9: Second filter

4.6 Second Segmentation

(see fig. 9)
[see first segmentation]

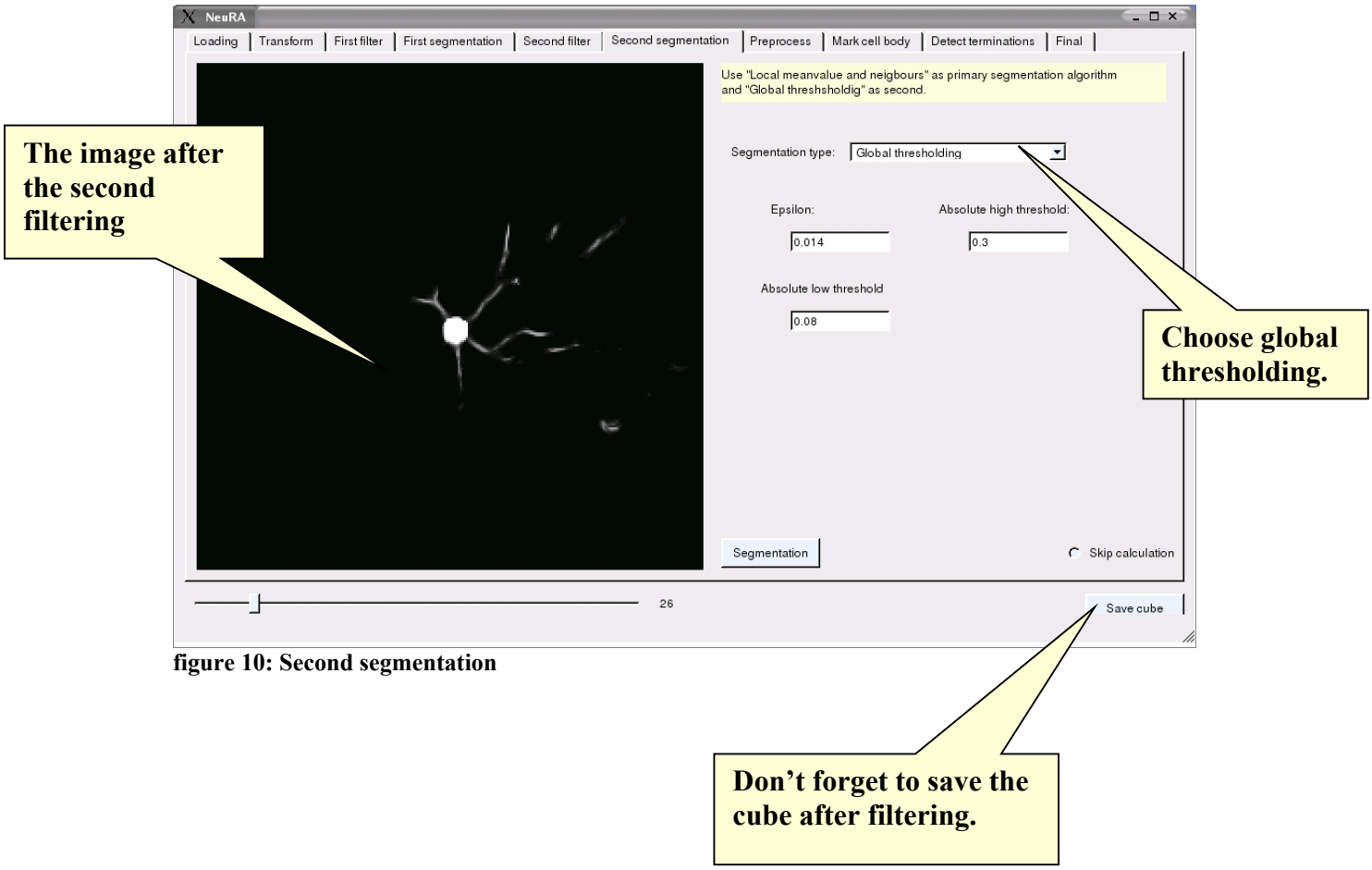


figure 10: Second segmentation

4.7 Preprocess

(see fig. 12)

Before starting the reconstruction, the image has to be preprocessed.
All structures which do obviously not belong to the neuron have to be marked and deleted.
This is the case for boundary artefacts and the pipet, which would otherwise disturb the algorithm.

Delete boundary: […]deletes artefacts on the boundary (= the next two voxels adjacent to the boundary]

Erase pipet: […] [erases a conus out of the image stack]

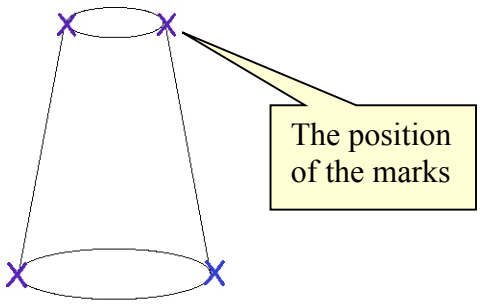


figure 11: Conus for erasing the pipette

Set four marks to erase the pipet. Two at one end and two at the other. (see fig. 11)

TIP:You will obtain the best result if you set the marks on a page of the image where you can see as much as possible of the pipet.

To set a mark, press <shift> + LMC, to remove a mark, press <ctrl> + LMC.

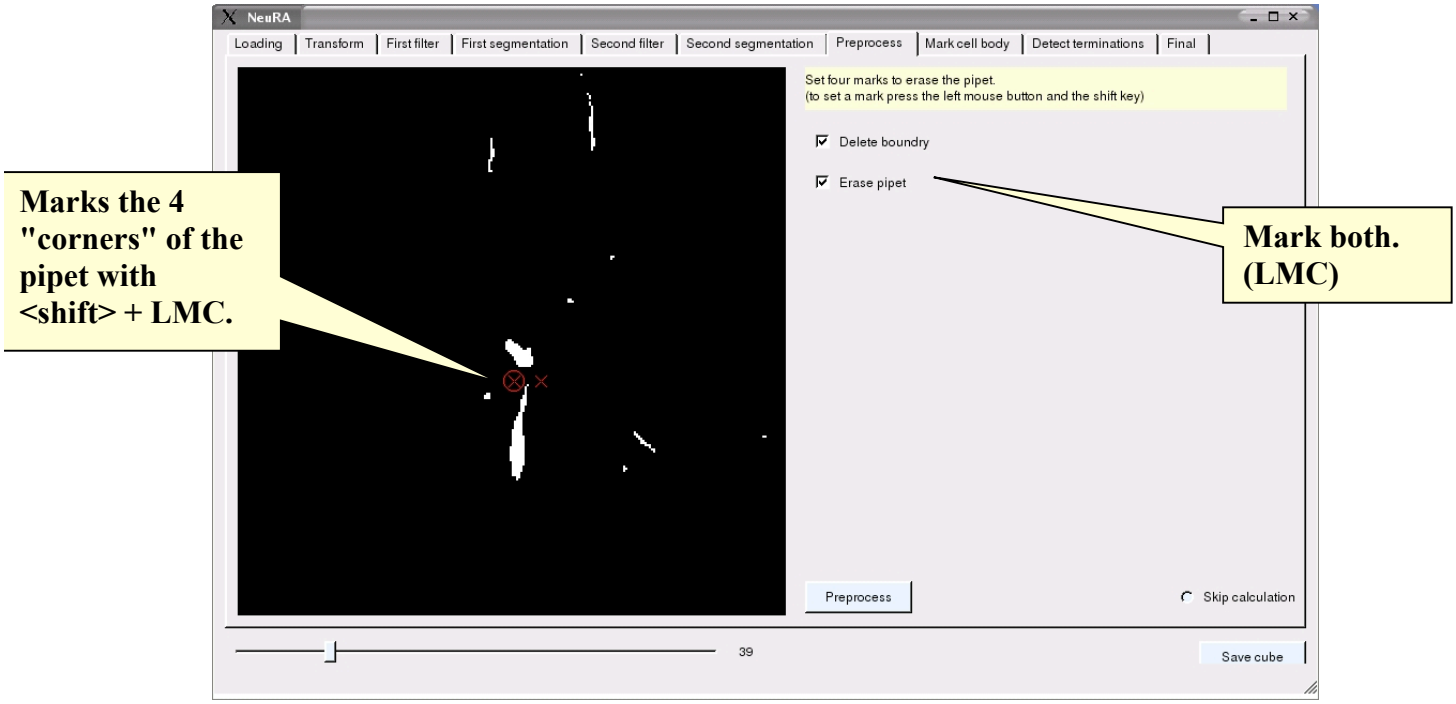


figure 12: Preprocess

4.8 Mark cell body

(see fig. 13)

The center of the cellbody is the starting point of the reconstruction. Therefore it is necessary to place the mark carefully in the center of the cell body.
To set a mark, press <shift> + LMC, to remove a mark, press <ctr> + LMC.

After pressing the *Process* button NeuRA will start to detect tree terminations.

TIP: To find the center of the cell body in z direction, zoom through the image and count the number of the pictures where the cell body is visible. Mark the cell body center in the picture in the middle between start and end number.

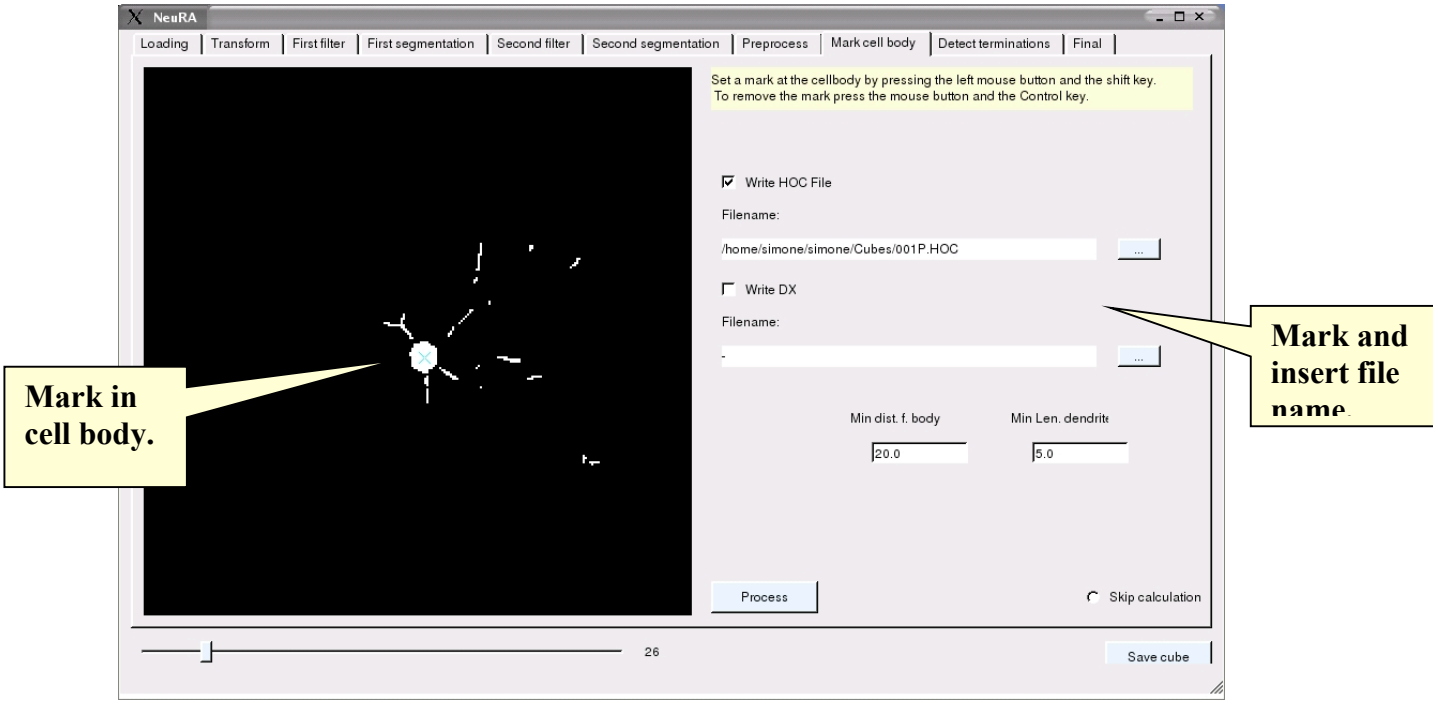


figure 13: Mark cell body

4.9 Detect terminations

(see fig. 14)

The marks (red cirles with a cross) show, where the algorithm has found dendrite terminations.
Detected structure is colored bright white.
Not detected structure is colored gray.
You can delete terminations the algorithm has found by moving the mouse cursor on the marked point and pressing <ctrl> + LMC.
You can add terminations in regognized structures with <shift> + LMC.

You also can decide which kind of cube shall be displayed on the next image screen: *Transformed, filtered, segmented, filtered and segmented, final, preprocessed or compplot*.

TIP: Zoom rapidly back and forth through the image to recognize contigous structures.

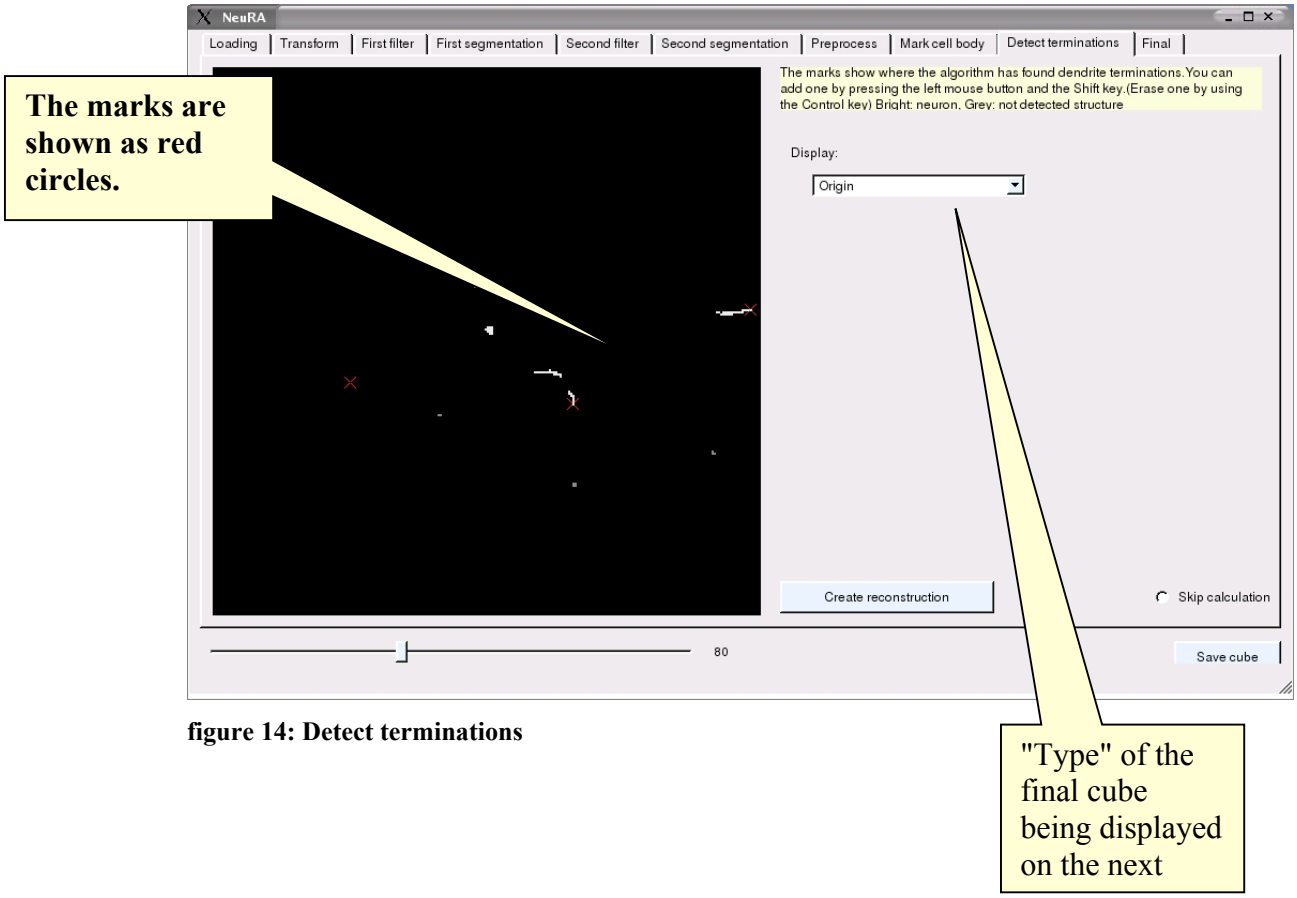


figure 14: Detect terminations

4.10 Final

(see fig. 15)

This plot shows you the final reconstruction.

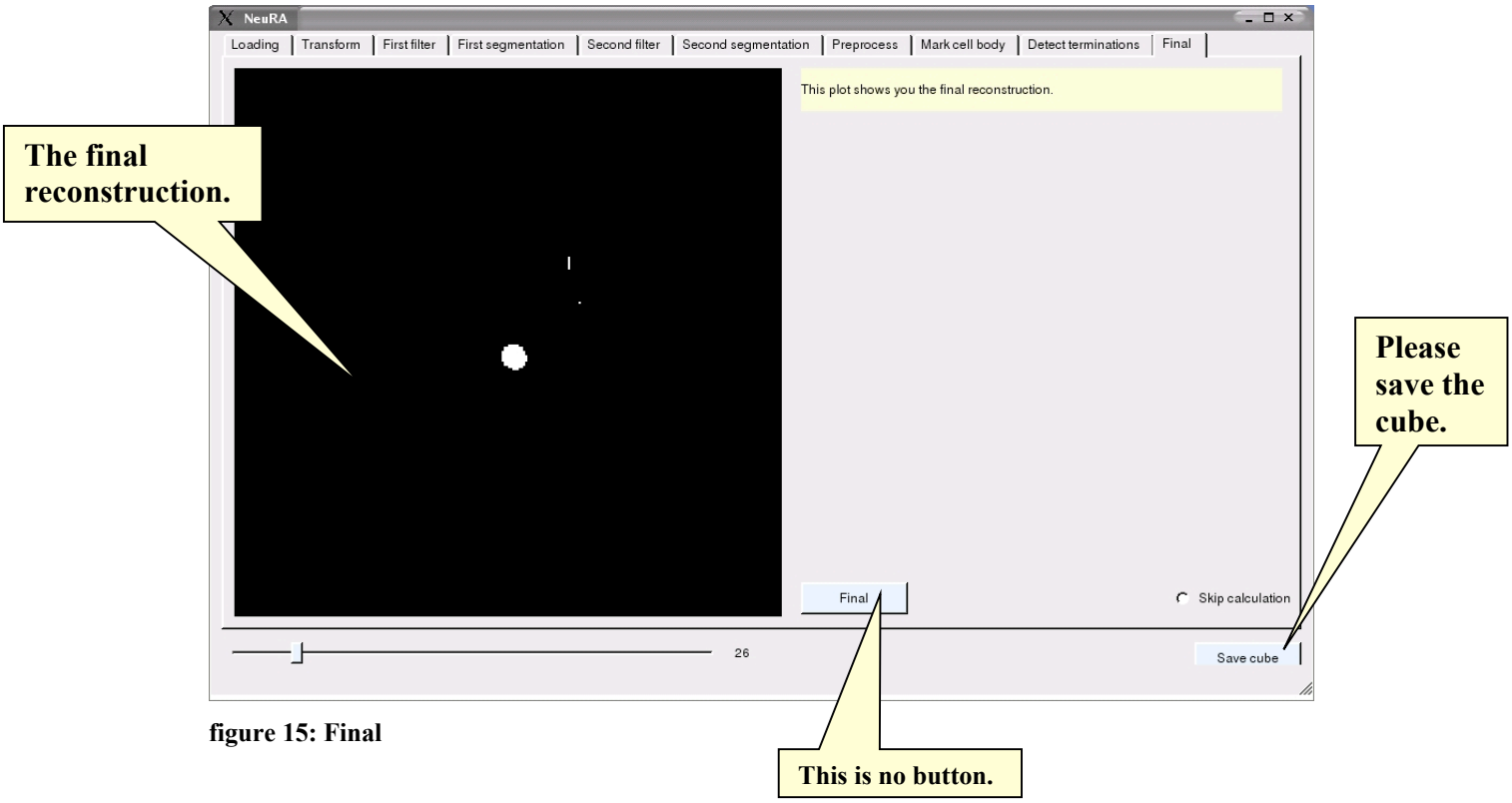


figure 15: Final

TIP: Don't forget to save the cube!