VASCULAR AUTO TRACING MANUAL

@ author: Yiming Kang
@ version: 05-14-2014

INITIALIZE AUTO TRACING PROGRAM

1. System Requirement:
   Mac OS X 10.6 is the recommended operation system.
   Matlab 2011a (or higher version) and Xcode 4 (or higher version) are required to be installed.

2. Program Directory:
   Folder "yk_VascularTracing" contains two Matlab .m files AutoTracing.m and Fixtracing.m and a folder of functions files.
   The default program directory is user/Documents/MATLAB/yk_VascularTracing.

3. Program Compiling:
   Run AutoTracing.m to compile mex function, enable parallel computing and initialize GUI panel. Wait till the following warning dialog (Figure 1) disappears and a GUI (graphic user interface) panel appears. The program is ready as the progress status in GUI panel indicates that "Parallel Computing Enabled; File msfm3d.c Compiled".

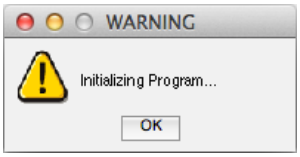

Figure 1. Warning dialog indicates initializing the auto tracing program.

* Troubleshooting *
If see error message like the following, please follow the instruction in this link (http://mathworks.com/matlabcentral/answers/94092).

```
/Applications/MATLAB_R2011a.app/bin/mex: line 305: gcc-4.2: command not found
```

OPERATION OF AUTO TRACING

The GUI panel (figure 2) controls all functions in data preparation, image segmentation, and auto tracing.

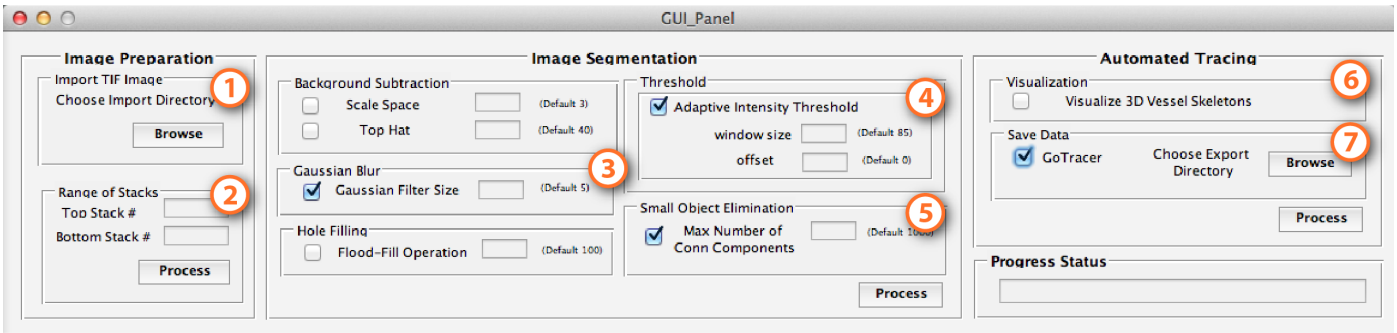

Figure 2. GUI Panel.

1. Click "Browse" to choose image stack to load. Note that the stack needs to be 8-bit (no 16 or 32-bit), grayscale (no RGB), and tiff format; otherwise, error message will pop up. After loading the image, the progress status will indicate the size of 3d image (width x height x stack number).
2. Enter the range of stack to be traced, and click "Process". Note if tracing cerebral image, the top stack is recommended to choose below the collagen fiber layer.
3. Check the checkbox for 3d Gaussian filter with default filter size of 5 pixels, and it's optional to enter a different filter size. The Gaussian filter reduces image noises and smooths the boundary of vessel objects.

4. Check the checkbox for adaptive intensity thresholding with default window size of 85 pixels and 0 intensity offset. It's optional to change window size and offset for better binarization result. This function yields binary data, in which the voxel value of 1 is vessel object and the voxel value of 0 is background.
5. Check the checkbox for small object elimination with default volume threshold of 1000 voxels. It's optional to change volume threshold. This function helps to further reduce image noises by eliminating the small cluster of connected-components with the size under maximum volume.
   Click "Process" to start image segmentation. A progress bar will pop out to indicate segmentation processing status. An image viewer window will pop out after segmentation finishes to give user slice-by-slice comparison of the original image and binary data (figure 3). If binary data is not optimal, user could tune the segmentation parameters and reprocess it.

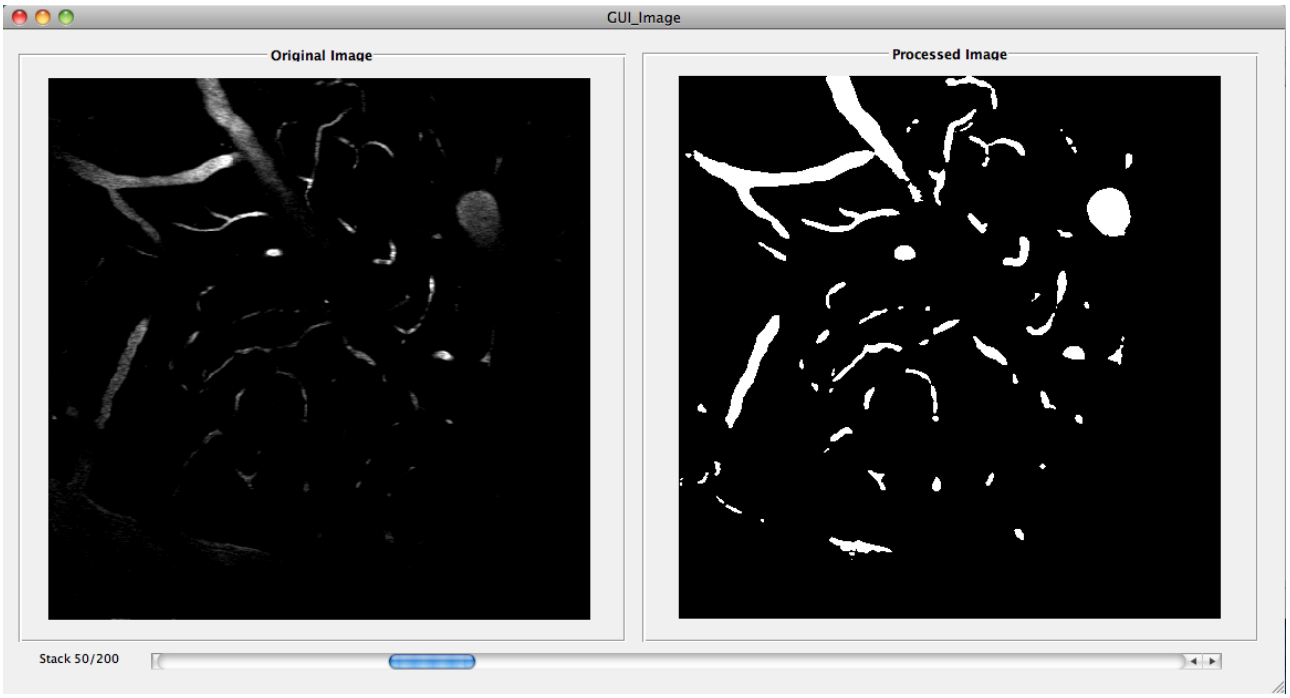

Figure 3. Image viewer for comparing original image (left) and binary data (right).

6. It's optional to check the checkbox for visualizing 3d vessel rendering after auto tracing (Figure 4).

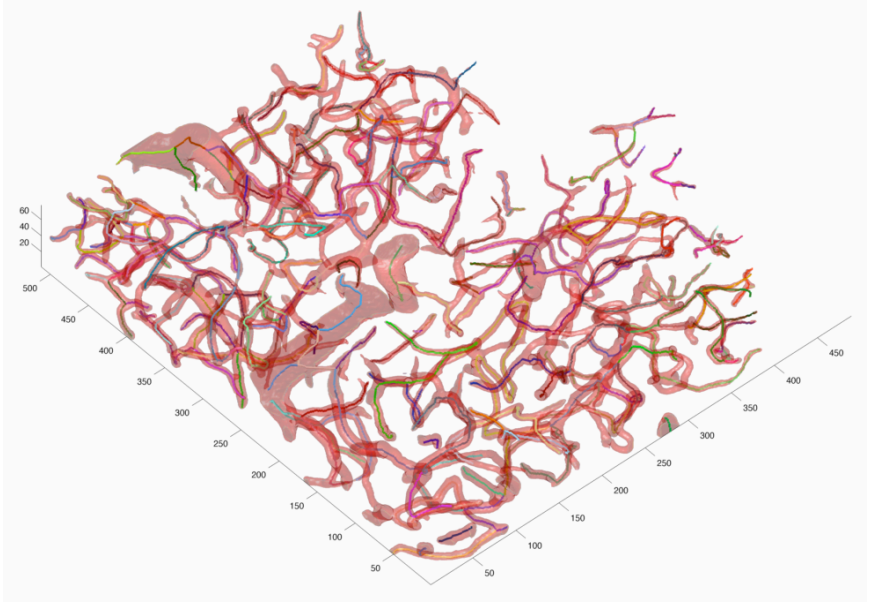

Figure 4. Rendering of vascular volume and tracing data.

7. Check the checkbox of GoTracer, and click “Browse” to choose directory and type in output filename to designate saving .mat and .gotrace files. And click “Process” to start auto tracing (Figure 5). Two warning dialogs indicates the status of auto tracing.

Figure 5. Warning dialogs indicate parallel auto tracing status.

TRACING DATA STRUCTURE

The traced vascular network (variable “SS” in output .mat file) is represented as a graph, containing nodes and edges. Each vessel segment has two nodes (the two ends of a curve) and one edge (the linepath of a curve). The node can be either the tip of a vessel segment, which has no connection to other vessels or the junction point where the vessel bifurcates.

More specially, in Matlab data, each entry in the "Nodes" with a unique index number represents a node as describe above. "Connections" denotes a list of edge indices this node connects to. "Locations" and "locationsInMicrons" are the node coordinates in image voxel unit and in microns respectively.

Each entry in the "Vessels" represents a vessel edge. Like what describes above, "Connections" denotes a list of node indices this edge connects to. "StartPoint" and "endPoint" are the two ends of this edge, whose order doesn't matter. "Linepath" denotes the coordinates of the discrete traced points that line along the vessel axis.

POST TRACING CORRECTION

Post tracing correction is a back-and-forth procedure to optimize vascular tracing using GoTracer and Matlab:
Fix tracing manually (GoTracer) >> Automatically fix tracing structure (Matlab) >> Verify tracing (GoTracer)

1. **Manually fix tracing in GoTracer**
   Open output .gotrace file yielded from Matlab auto tracing program along with its corresponding image stack. Go to “Format >> Dimensions >> Show Dimension Options”, and set micron per pixel and micron per layer.

   1.1. There are currently 3 scenarios of correcting auto traced data.
   Scenario a. Lost connection of vessel linepath to node.
   Solution: Nothing needs to be done in GoTracer. Matlab program will fix it automatically (Figure 6).

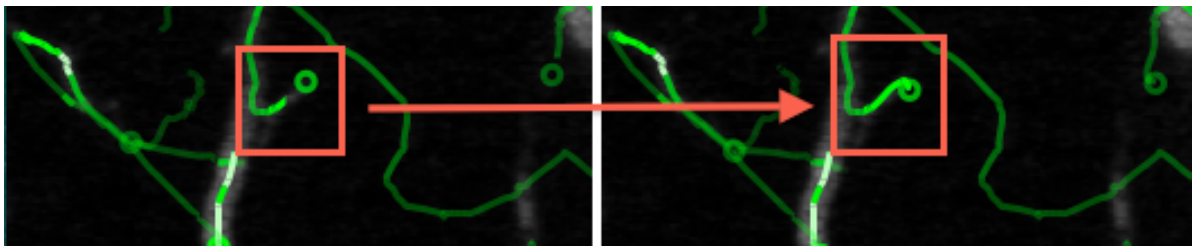

Figure 6. (Left) Lost connection. (Right) Connection fixed through Matlab program.

Scenario b. Gap between two disjoined vessel segments that were supposed to be connected.
Solution: Directly trace from one node to the other to fill the gap. Those three segments will be merged in Matlab to be one intact vessel segment (Figure 7).

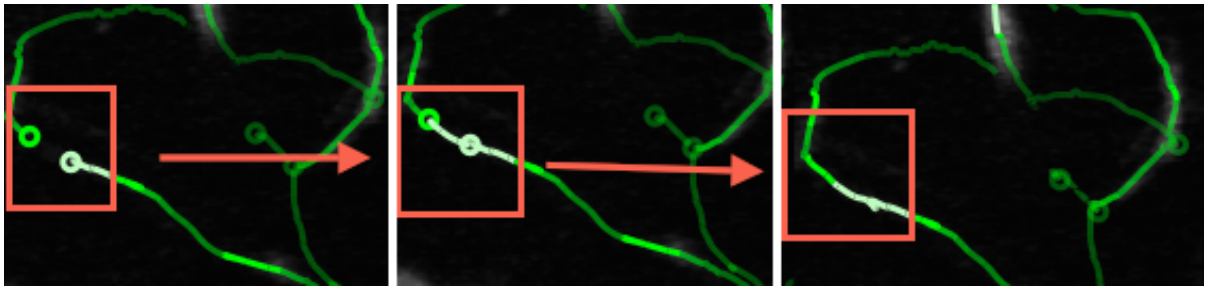

Figure 7. (Left) Gap. (Mid) Trace new segment based on existing nodes. (Right) Merged as one through Matlab.

Scenario c. Lost connection between multiple vessel segments that are not at the tip of segment.
Solution: Add node (bifurcation node) on the existing segment to split it into two segments. Mark the node as "Communication Capillary" so that Matlab will recognize this new node. Trace new segment based on new nodes (Figure 8).

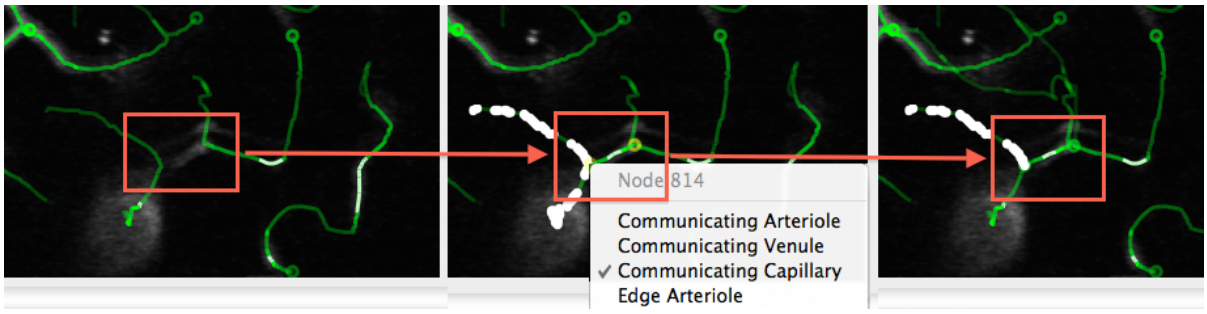

Figure 8. (Left) Gap. (Mid) Add new node on the segment that needs to be split, and trace new segment. (Right) In this case, Matlab splits both original vessel segments and creates 5 segments connectivity.

1.2. When tracing new vessel segment, make sure place starting and ending linepath points close to the corresponding nodes (<5px) (Figure 9).

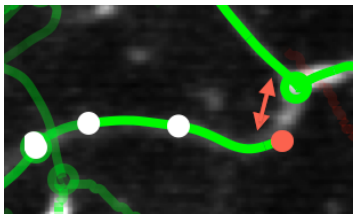

Figure 9. This example shows segment too distanced away from node for Matlab to correct.

1.3. Mark potential ambiguous crosses. Reference the original image stack or the stacks taken in different imaging dates to resolve.
1.4. Keep tracing all intersections towards the image boundary (Figure 10). Boundary bifurcation information is also important for topology analysis.

Figure 10. A good example of tracing towards image boundary.

1.5. Make sure to mark the surface arteries and veins. Only mark the “elbows” as penetrating arterials and venules. “Elbow” is defined as the segment connected to surface arteries or veins, while penetrating or ascending through the tissue, and stops at the first bifurcation node (Figure 11).

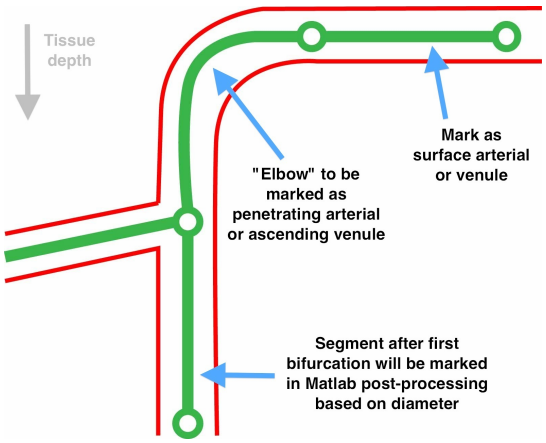

Figure 11. Notation illustration.

2. Fix tracing structure in Matlab

2.1. In GoTracer, go to “File >> Export to >> MATLAB..” and export as .plist file.

2.2. Run FixTracing.m under the same directory as AutoTracing.m program in Matlab. Load both the .plist file and the corresponding image stack. The program will automatically save the fixe vascular structure in .m file and .gotrace file after tracing is fixed.

3. Verify tracing in GoTracer