```
root:bNV:gUserName="Micek"

root:Hyperstack:gHyperstackWindowSize="Small"

#root:Hyperstack:gHyperstackRootHDD="Work:jhu:Hyperstack:"
root:Hyperstack:gHyperstackRootHDD="d:Users:cudmore:Hyperstack:"

#root:Hyperstack:gHyperstackRawRepo="Work:jhu:hyperstack:rawRepo:"
root:Hyperstack:gHyperstackRawRepo="d:Users:cudmore:hyperstack:rawRepo:"

#root:Hyperstack:gDatabasePath="Work:jhu:hyperstack:intermediate_hyperstack:micek:"
root:Hyperstack:gDatabasePath="d:Users:cudmore:hyperstack:micek:"

root:Hyperstack:gLoadAndPlotOnLoad=0

#root:bStack:gIAmSuperuser=1

#micek
#gHyperstackRootHDD = "d:Users:cudmore:Hyperstack:"
#gDatabasePath = "d:Users:cudmore:hyperstack:micek:"
#gHyperstackRawRepo = "d:Users:cudmore:hyperstack:rawRepo:"
```

foobar

How do I move a node or slab?

To move an object, a node or a slab you
1. select the object you want to move (it will turn yellow)
2. move to the imaging plane it will go to
3. right-click the object and select the 'Move…' menu. You will see a red crosshair on the object.
4. drag the object to the new x/y position (don't change the imaging plane)
5. click away from the object to commit

I ran out of memory?

Stacks take up a lot of memory. When a stack window is closed it is still in memory. Unload unused stacks in the main Hyperstack panel with 'Unload Stacks'.

My stack window is not behaving correctly?

Close and re-open the timepoint.

My key-presses are not doing anyhting?

Make sure 'Caps Lock' is not on

Where are my files?

d:\Users\cudmore\hyperstack\intermediate_hyperstack

How do I copy a hyperstack from one computer to another?

You need to copy two things (i) the hyperstack file and (ii) the hyperstck folder
For a hyperstack named a153*hs2, you need to copy:*
*File: a153*hs2*T.ibw*
*Folder: a153*hs2

bHyperstack is VERY particular about the placement of files on the hard-drive

Do NOT change the names of files on the hard-drive by hand

When you open a stack from hard-disk, give it some time.

If you jump right in and start clicking before the stack is fully loaded you may get errors. This usually happens when loading a timepoint with the 'Stack' button in the main hyperstack window. It also happens when opening two timepoints from the Find Points interface.

Selected objects including nodes, edges and slabs are not masked based on the visible imaging plane, they are visible in all imaging planes.

This can be confusing.

Creating slabs on tubes that are coming in or out of the imaging plane can be a nuisance.

The slabs visually overlap. You will get the hang of it.

Saving a hyperstack can be slow.

The program saves all timepoints every time you click 'Save'.

How to do some things in [Jekyll](#)

As of Feb 2015 I discovered Jekyll and this has rocked my world. Beautifully done. No more Word-Press for me !!!

Install on OSX

```
sudo gem install jekyll
```

Making posts and pages

To make a blog post, the blog-post.md file needs to start with this yaml [front matter](#)

```
---
layout: page
title: "example user file"
category: post
date: 2015-01-30 22:01:06
---
```

To make a new page new-page.md the starting yaml front matter looks like

```
---
layout: page
title: "Opening and Closing Igor Pro"
category: gettingstarted
date: 2015-01-30 22:38:12
order: 3
---
```

Making external hypertext links

Reference links look like this

```
This links to [Jekyll front-matter page][1]
This links to [Daring Fireball][2]

[1]: http://jekyllrb.com/docs/frontmatter/ "Jekyll Front-Matter"
[2]: https://daringfireball.net/projects/markdown/basics "Markdown Bascis"
```

Internal Links

I edited [_config.yml](#) like this

```
# Dates are not included in permalinks
#permalink: none
permalink: /:title
```

I do not need to add anyhting special to my yaml frontmatter!

And now this works as link to [stack](#) this works as link to [stack](#)

```
this also works, [Link to stack page][3]
[4]: {{site.baseurl}}/stack/ "stack"
```

this also works, [Link to stack page](#)

On the mac

I am keeping my local Jekyll sites in /Users/cudmore/Sites/Vascular-Analysis

To run Jekyll on a Mac,

```
cd /Users/cudmore/Sites/Vascular-Analysis
jekyll serve --watch
```

View the site locally with

```
http://localhost:4000/Vascular-Analysis/
```

View the site on Github

```
http://cudmore.github.io/Vascular-Analysis/
```

Convert markdown to pdf

```
cd /Users/cudmore/Sites/Vascular-Analysis/_posts
//with TOC
gimli -y -m -s ../css/main.css -w '--toc --footer-right "[page]/[toPage]"' -o pdf
//without toc, -w is passing commands to backend wkhtmltopdf
gimli -y -m -s ../css/main.css -w '--footer-right "[page]/[toPage]"' -o pdf
```

gimli -y -m -s ../css/main.css -w '--footer-right "[page]/[toPage]"' -o ../pdf

gimli -y -m -s ../css/main.css -w '--header-left "[webpage]" --header-right "[page]/[toPage]"' -o ../pdf

Hyperstacks are loaded, saved, and visualized through the Hyperstack panel.
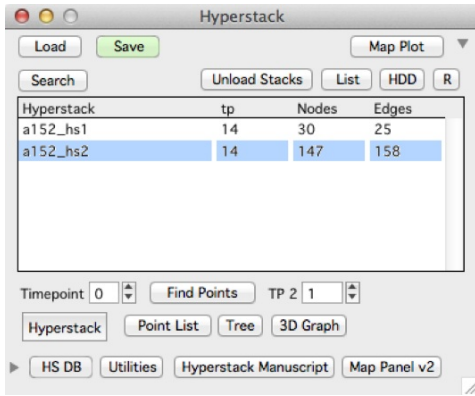
Loading a hyperstack

Press the 'Load' button and select the _T.ibw file corresponding to the hyperstack to be loaded.

If the hyperstack to load is a153*hs2 then load the file 'a153*hs2_T.ibw'

Saving a hyperstack

- Select a hyperstack in the list
- Click the 'Save' button
- The hyperstack is saved in the same hard-drive folder it was opened from.

Opening a **stack** window for a single timepoint

1. select a hyperstack in the list
2. fill in the timepoint
3. click 'Stack' button.

Opening **Find Points** to connect nodes and edges from one timepoint to the next

- Enter two sequential timepoints in 'Timepoint' and 'TP 2'
- Click 'Find Points' button
- See Find Points

Notes

- Only one timepoint can be viewed at a time with the 'Stack' button.
- There is no 'Save As...'

> **Tip:** If you lose the hyperstack panel, it can be reopened with menu
> Hyperstack -> Hyperstack Panel

This vascular analysis code is currenlty being used in the Linden lab. If you are interested in what you have found here, please email robert cudmore.

foobar

This vascular analysis runs inside of [Igor Pro](#).

Open bHyperstack in Igor Pro

- Double-click on desktop icon bHyperstack.
- Inside Igor, activate the Hyperstack menu by clicking on the Igor command window.
  The Igor command windows title is 'Untitled'.
- Select menu 'Hyperstack -> Load User File' and select your .txt file.

Load a hyperstack

- In main [Hyperstack panel](#), click 'Load' button.
- You then load a hyperstack by opening a file ending in _T.ibw
- For example if your hyperstack is named a152 *hs1, you would load the file 'a152* hs1_T.ibw'

Save a hyperstack

- Saving is done one hyperstack at a time.
- Select a hyperstack in main [Hyperstack panel](#) and click 'Save' button.
  The hyperstack will be saved on the hard-drive in the folder it was loaded from.

Quit Igor

- Select menu 'Igor Pro -> Quit Igor Pro
- You will be asked 'Do you want to save changes to experiment "Untitled" before quitting'.
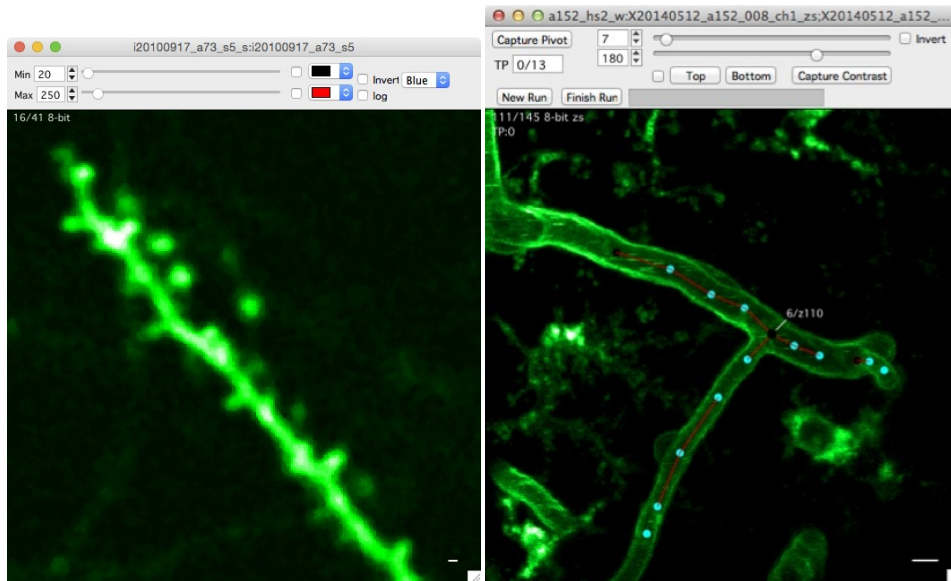 Answer No.

> **Important:** You are responsible for saving your work for each hyperstack. When you quit Igor Pro, your work is NOT AUTOMATICALLY SAVED.

You save your hyperstack using the save button in the main [hyperstack panel](#).

Each timepoint is a 3D volume (a stack).

Examples

Two example stacks. Dendritic spines (left) and vascular tubes (right).

Interface

- Mouse wheel to scroll up and down through images
- Image contrast is critical, set it with the sliders. Oen and close contrast bar with keyboard 'c'
- View a histogram with keyboard 'h'

Keyboard commands

```
Arrow keys to pan left/right and up/down in the image
+/- keys to zoom in/out
Return/Enter keys to zoom all the way out

Left mouse button to select objects
Esc : Cancel selection

1 : view channel 1
2 : view channel 2

3 : view a sliding maximal z-projection of channel 1
4 : view a sliding maximal z-projection of channel 2

c : toggle contrast control bar
h : open histogram window
] : toggle between two different window sizes

sift+c : cycle through 4 different window 'candy' displays

shift+p : Set pixel/voxel size

shift+? : open the 'Point Info' window
    Object notes can be set in the Point Info window.
```
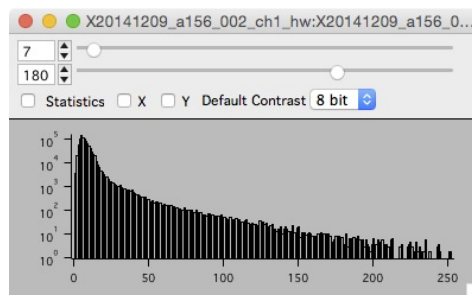
Histogram

- Open the histogram window with keyboard 'h'.
- The histogram window shows a pixel intensity histogram for one image.
- Scroll through the image and the histogram for each image will be show.

Navigation window

- Right click and select 'Navigation Window' menu. A navigation window will be opened and your current zoomed view of the stack will be shown as a blue square.
- Zoom with +/- or pan with arrow keys and the blue-square tracks what you are looking at.
- The navigation window is, by default, a maximal z-projection of the entire stack.
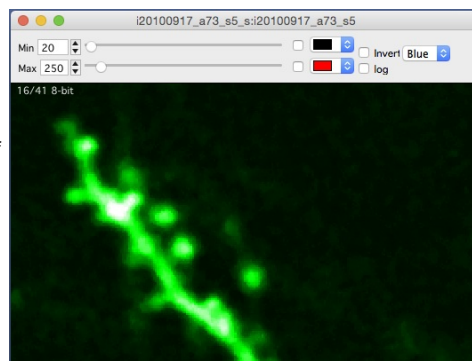
Point info

The Point Info window shows information about the currently selected object.

Open the Point Info window from any stack window using shift+?.

Use the point info window to annotate either nodes or edges with notes.

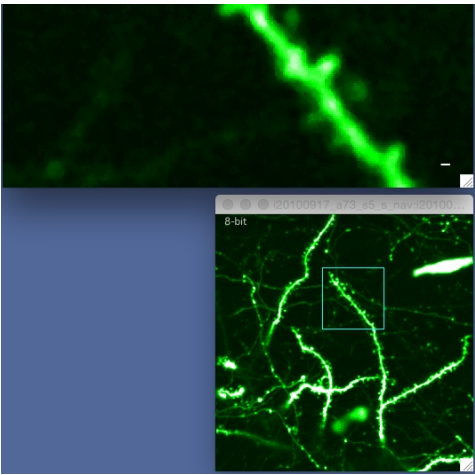Use the point info window to annotate edges as 'diving', 'surface', 'Has Pericyte' and 'Branch Order'.

Notes

- Closing a stack window does not remove the stack from memory.

(1) Make nodes, tubes and slabs in one timepoint

- See [Vascular tracing](#) for more information
- Create a node at one vascular branch point (shift+click)
- Create another node at the other end of the same tube (shift+click)
- Create a tube by selecting the first branch point (source) then N+click the second branch point (destinaiton).
- Create slabs along the tube with S+click

Check each slab diameter for each new tube

- Once all slabs for a tube are done, review the slabs (and their radii) using left/right arrow keys. Fix slabs with bad radii using the [editing slabs](#) interface

Keep in mind

- If marking a subset of tubes, only mark tubes where you would put more than one slab, e.g. don't score super short tubes.
- If marking a subset of tubes, keep an eye on the depth (image plane) of your tubes. You should only mark tubes that are in and around layer II/III somas. Use keyboard '2' to switch to channel 2, the somas appear as black dots.
- If a tube has a pericyte, try and get a slab centered on it. If this is not possible, specify 'has pericyte' for the edge using the [Point Info](#) window.
- Annotate your images (using node and tube notes) with anything that is out of the ordinary or just cool.

(2) Make similar nodes, tubes and slabs in the next timepoint

- You can open sequential timepoints using the [Find Points](#) window

(3) Connect nodes from one timepoint to the next using [Find Points](#) window

- Open FInd Points for two consecutive timepoints, e.g. timepoint 4 and 5.
- Selecting a row in the list in Find Points will open two stacks, snap the images to the same region and highlight the selected node (if they are connected).
- Use keyboard arrow keys in the Find Points list to scroll through nodes. Keyboard left will flash the programs best guess, Keyboard right will flash the actual connection.
- Keyboard shift + right-arrow will transfer the Guess to the Dst.
- If the guess is not to your liking, select the nodes you want to connect (in their stack windows), right-click on the desired destination node and select 'FindPnt -> Dst' menu.

(4) Quality control

- Check all slab diameters using [search](#) 'All Slabs'. Scroll through the list of slabs for a timepoint and the slab selected in the list will be selected in the image

(5) Marking tubes as diving, surface, has pericyte and marking branch order

- See [Scoring tubes](#) for more information.
- In one timepoint, mark all tubes as diving (keyboard 'd') or surface (keyboard 's'). If a tube is neither, leave this blank.
- In the same timepoint, mark the branch order of each tubes 0..9. If you can't determine the branch-order of a tube, leave it blank.
- Mark tubes with pericytes (keyboard 'p')

20150109

Finally getting around to some bug fixes

New Features

1) edges now have notes. use info window, 'i' from main hyperstack window

2) can now search for edges and slabs (previously search was limited to nodes) we will use this to step through all slabs and edit radius/pericyte

3) can now mark a slab as pericyte in main hyperstack window, keyboard 'p' in slab search result list, keyboard 'p' in slab diameter panel, with checkbox

Bug Fixes

- fixed moving cursor bug when turning on 'manual' connect.
  see: print "20150109, removed call to SlabRadius_SetZ() to fix 'manual' bug. May cause errors"
- decided to NOT fix extra slab when user clicks left-mouse and are no longer holding down 's'
  workaround is to just hit 'esc'
- entering out of bound timepoints will now warn when displaying a hyper stack stack window or FindPnt
- we no longer warn when switching between auto/manual slab
- temporary one liner to fix color of nodes (is incorrect defaulting to black)
  $MColors[1][0]=65535;M$COlors[1][1]=49151;M_Colors[1][2]=49151

20150123

New Features

1) revamped the 'search' panel. - can now search for different kinds of objects: nodes, edges, slabs - added feedback on last search type (node/edge/slab) and the number of hits - better system for opening stacks (and stack runs) when an object is selected in the search results

2) edges now have isDiving and isSurface values - To set Diving and/or Surface, select an edge and hit 's' to toggle 'isSurface' and 'd' to toggle 'isDiving' - These keyboard command will work for an edge selection in search results - ToDo: these keyboard commands will work in the main hyperstack window (problem is that 's' is already mapped to making a new slab)

Minor Improvements

- when loading a hyperstack and it is already loaded, we will only ask you once and if you accept we will OVERWRITE the already loaded hyperstack Previously, we would ask to verify loading each timepoint
- The main Hyperstack panel will always default to timepoint 0 and TP 2 1.

20150129 Henri started

New Featurues

- changed system of delete for slab and node. Now just select object and hit 'Delete'
- changed system of move for node and slab. Now just select object and hit 'm' Next click will be the new position (including z) Esc will cancel out and not move

Minor Improvements

- main hyperstack panel now has interface to edit raw repo path (use expansion triangle) next step is to get some of student scoring data onto solid-state-drive (requires swapping rawRepo)
- stepping forward/back to next slab with left/right arrow keys now stops you at the enf the edge (at the lst slab)
- manual slab cursors are now red

All three types of object (nodes, edges and slabs) have a similar interface to select, create, move and delete. All objects are placed in x/y and z where z is the currently viewed imaging plane.

Nodes (Nodes are vascular branch points)

- Select: left-click (will turn yellow)
- Create: shift+click
- Move: select and press keyboard 'm', next click will be new posiiton
- Delete: select and press keyboard 'Del'
- Notes: Use the Point Info window (open with shift+?)

Edges (Edges are vascular tubes that connect one branch point to another)

- Select: left-click a slab within the edge
- Create: Making a new edge is a two step process
  (1) select the source node
  (2) N-click the destination node (a line will be formed between them)
- Move: Edges cannot be moved
- Delete: right-click and select 'Delete Edge'
- Notes: Use the Point Info window (open with shift+?)

Slabs (Slabs are the actual tracing along a vascular tube)

- Select: left-click
- Create: S-Click
- Move: select and press keyboard 'm', next click will be new posiiton
- Delete: select and press keyboard 'Del'
- Notes: Slabs do not have notes

  Once a slab is selected you can scroll to the next/previous slab using keyboard left/right.

  It is sometimes hard to select a slab if it is not in the currently viewed imaging plane. Scroll the image up or down a bit and try again.

All objects are placed in x/y and z. Where z is the currently viewed imaging plane.

Only one edge between any two branch points.

When a node, edge, or slab is selected it will be visible in all imaging planes, not just the currently viewed imaging plane.

Aligning timepoints

The beautiful thing is we do not align timepoints in the traditional sense, there is no need to modify your original .tif image files.

Alignment is done by specifying a 'pivot point' that is common to all timepoints to be aligned.

Fitting tube diameters

Tube diameters are fit as threshold crossing at the full width at half maximum ([FWHM](#)).

Sliding maximal z-projection

This is used to visualize slices above and below the current imaging plane. Please see my main website, [here](#), for more information.

foobar

Each slab has a fit to the tube diameter. It is critical that this fit is correct. Most fits are correct (~90%) but the remaining bad fits need to be edited by hand.

To manually set the radius:

- Switch to 'Manual', will show cursors on the intensity line in the 'Slab Radius Panel'.
- Drag the cursors to the correct positon, the red dots in the image will give you feedback.
- Click 'Commit'.

Capture Pivot   7   [ ] Invert

TP 2/28   110

[ ] Top   Bottom   Capture Contrast

New Run   Finish Run

112/267 8-bit
TP:2 Edge:22

The Find Points window will display two timepoints simultaneously and provides an interface to manage the connections between both nodes and edges across timepoints.

Open Find Points from the main [Hyperstack Panel](#) by specifying 'Timepoint' and 'Timepoint 2'. Select a row in Find Points and both the source and destination stacks will be opened.

When you first click a node row in the Find Points window, you are opening two image stacks. This will take some time. Let the program finish opening the stacks before you continue interacting with the interface.

slab diameter (um)

0   5   10   15um
slab index

Interface

The Find Points window displays a list of nodes in the source timepoint (Src column). For each node in the source timepoint, Find Points shows the corresponding connected node (if there is one) in the second timepoint (Dst column).

The Guess column shows the best guess for the corresponding node in the second timepoint. This guess uses each timepoints [Pivit point](#).

Once a node is selected, the keyboard can be used to scroll through the nodes in the Find Points list and nodes will be selected simultaneously in both the source and destination image stacks.

- Up arrow : Scroll to the previous node
- Down arrow : Scroll ot the next node
- Left arrow : Flash the Src node (in first timepoint) and Guess node (in second timepoint).
- Right arrow : Flash the Src node (in first timepoint) and Dst node (in second timepoint).
- shift + right-arrow : Transfer the Guess node to Dst node
- Del : Delete the Dst node (not the node itself, just the correspondence)

Nodes

foobar
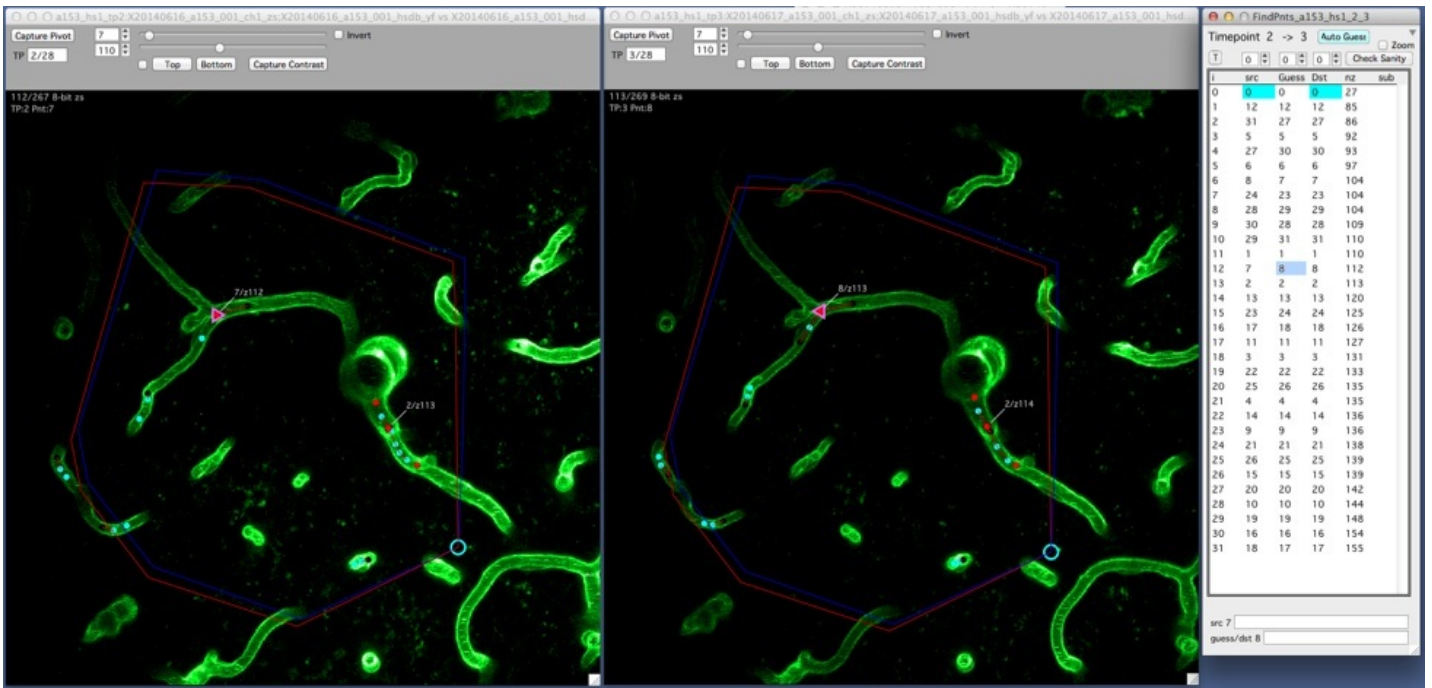
Find Points for nodes with Src node 7 connected to Dst node 8.

The goal is to fill in the Dst column with the correct nodes, e.g. a mapping of each node in the Src timepoint with a node in the Dst timepoint.

Find Points will show you a list of nodes in the first timepoint (Src) with their corresponding connection in the second timepoint (Dst).

The 'Guess' column is the programs best guess for which nodes are connected. This guess is created using Pivot Points.

Scroll through each source node and do the following:

- If the Guess is correct, transfer the Guess to the Dst by selecting the row in Find Points and using keyboard 'shift + right-arrow'. That is 'hold shift and hit the right-arrow'.
- If the Guess is not current, select the source node and the destination node to connect (in their respective image stacks) and right-click on the destination node (still in the image stack) and select menu 'FindPnt -> Dst'.
- Use 'Del' key to remove a node from the Dst column.

If a node in the second timepoint does not have a match in the first timepoint it is appended to the end of the list. This node can be selected as usual and allows for a reverse lookup from the second timepoint to the first.

Edges
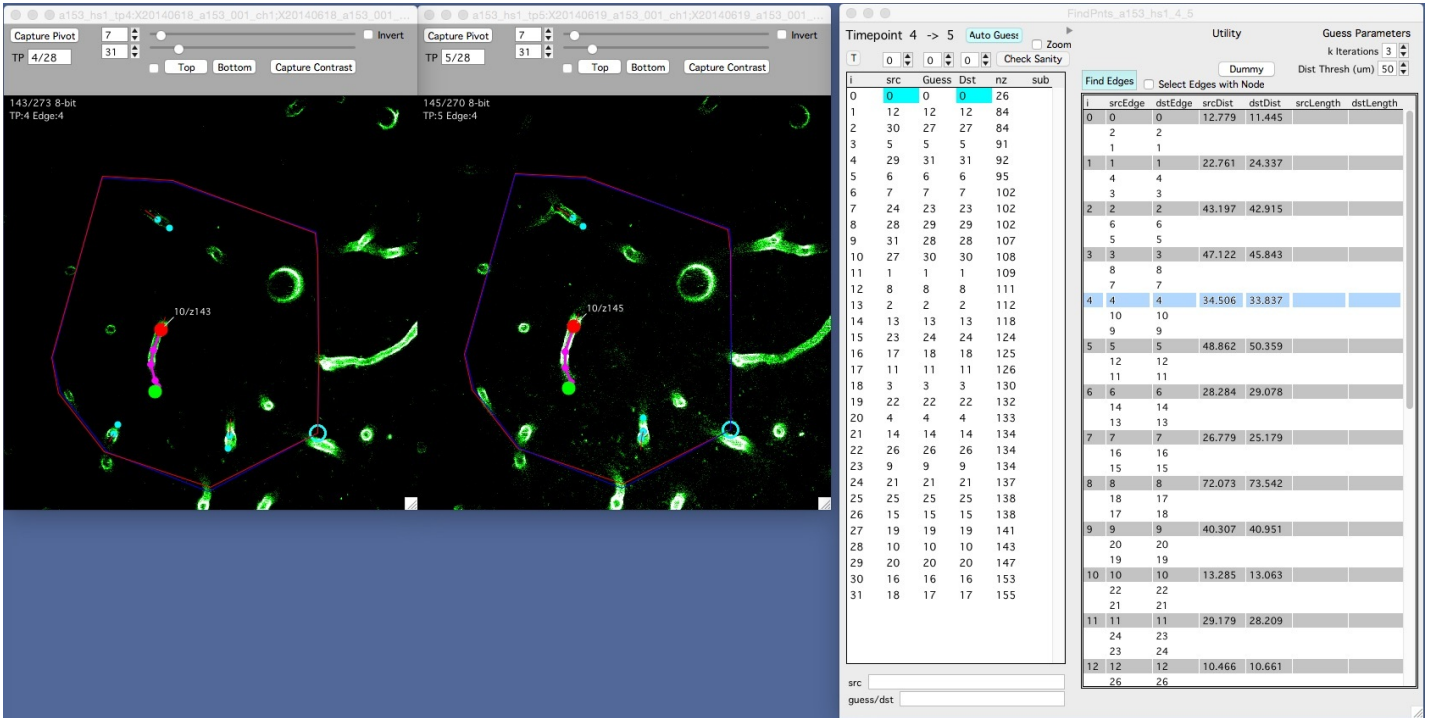
Find Points for edges with Src edge 4 connected to Dst edge 4.

Expand the FInd Points window with the disclosure triangle (top right of the window) to see a similar list for edges.

Edges in red are edges that have a mismatch between their starting and ending nodes.

You don't actually edit the connections between edges, you edit the connections between nodes and the edges will follow. This works because we only allow one edge between and two nodes (think about it).

foobar

Goal is to modify your node connecitons until the edges are as good as they can get.

foobar

The search panel will search for nodes, edges and slabs. It will search over all open hyperstack, just one hyperstack and across multiple timepoints or just one timepoint.

Open the search panel from the main hyperstack panel

Once a search is performed, clicking on a row in the results will bring up that object in a stack.

Pivot points allow 3D image stacks to be aligned from one timepoint to the next.

Any node can be a pivot point. Select the node and press 'Capture Pivot'.

The pivot point will only align stacks whose nodes are connected (see Find Points for how to connect nodes together).

If the guess in FInd Points starts to be wrong, pick a pivot point near the nodes you are working on and the Guess should get better.

Put another way

For each source node we generate a guess node using a pivot point in the two images. A pivot point is a special node that the user identifies as being the same in both images. By using the 3D position of this pivot, we can search from the source image to the destination image and look for nodes near that pivot (the closest match becomes the Guess). This allows for the actual registration of your image stacks to be fairly innacurate/bad from one timepoint ot the next. This begins to fail if the density of the nodes is high, we have trouble deciding who is actually the connecting node. This also starts to fail is there is a lot of angular rotation between sequential timepoints. This final problem can be greatly reduced by moving the pivot point near where you want to get a good Guess.

See Also

Find Points, Workflow

The Point Info window shows information about the currently selected node and edge.

Open the Point Info window from any image stack using shift+?.

Use the point info window to annotate either nodes or edges with notes.

Use the point info window to annotate edges as 'diving', 'surface', 'Has Pericyte' and 'Branch Order'.

foobar

Introduction

We want to add some more annotation to each vascular tube. We will be scoring each tube to describe:

1. If it is a diving artery/vein
2. If it is a surface tube
3. Its branch order off a main diving artery/vein (the actual diving arteries/veins get branch order 0)
4. Whether or not the tube has a pericyte.

Because your tubes are connected across timepoints using Find Points we can annotate tubes in **just one timepoint** and assuming the tubes don't change (with respect to these annotations) we can generalize these annotations across all timepoints.

You want to choose a timepoint for this new tube annotation that is early on and still easy to see. Just don't use the first timepoint.

Scoring tube properties

The main hyperstack stack window now has a 'Score Tubes' checkbox. When the 'Score Tubes' checkbox is on and you select an edge, the stack window will respond to the following keystrokes:

- d : Diving (will turn off Surface)
- s : Surface (will turn off DIving)
- p : Has Pericyte
- 0 : Branch order 0 (use this for diving arteries/veins)
- 1 : Branch order 1
- .
- .
- .
- 9 : Branch orer 9
- Del : Clear branch order

If you cannot determine the branch order of a tube, you want the branch order to be empty. Use keyboard 'Del' or just blank out 'Branch Order' in the 'Point Info' window.

You can also bring up the Point Info window (use keyboard shift+?) and set these values using check boxes for Diving, Surface and 'Has Pericyte' and a numerical field for 'Branch Order'.
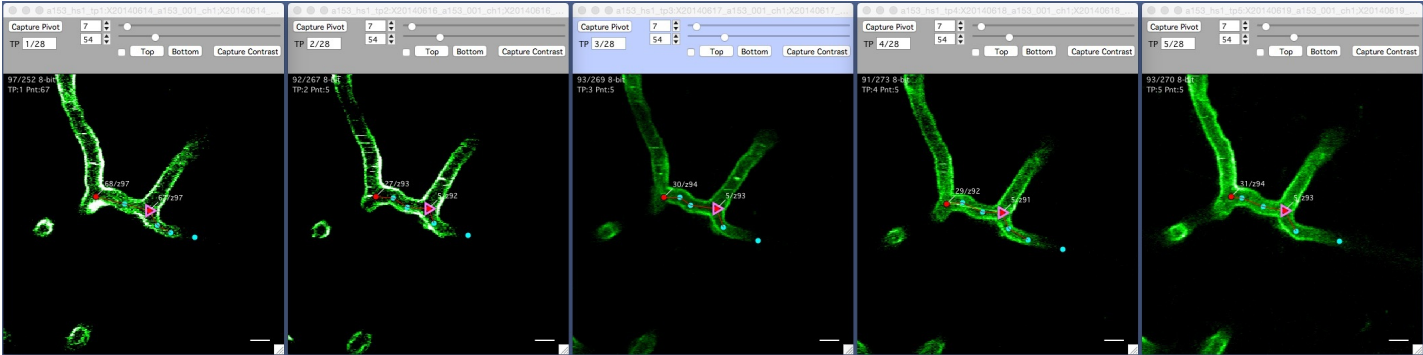
How to get started

- Open a stack window
- Turn on 'Score Tubes' checkbox
- Open point info window (shift + ?)
- Select a tube
- Try out some key strokes: d, s,p, 0, 1, 2, 3, ...

- See how the annotation changes in the point info window

- You want to make sure you get all your tubes annotatd. Clicking around on different tubes in the stack window is not that reliable (you might miss a tube). Use the Search window to generate a list of all tubes and go through each tube one-by-one.

A run follows an object (a node or edge) through multiple timepoints.

The easiest way to open a run is to click on an object in the [Search](#) window.

Node Run

Edge Run