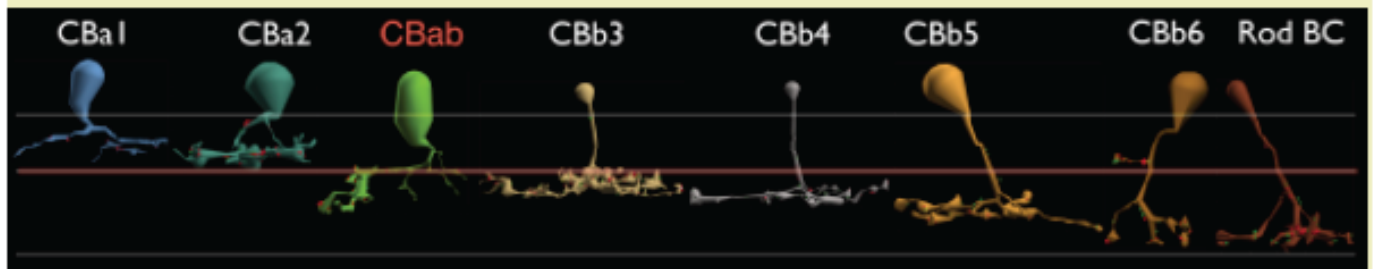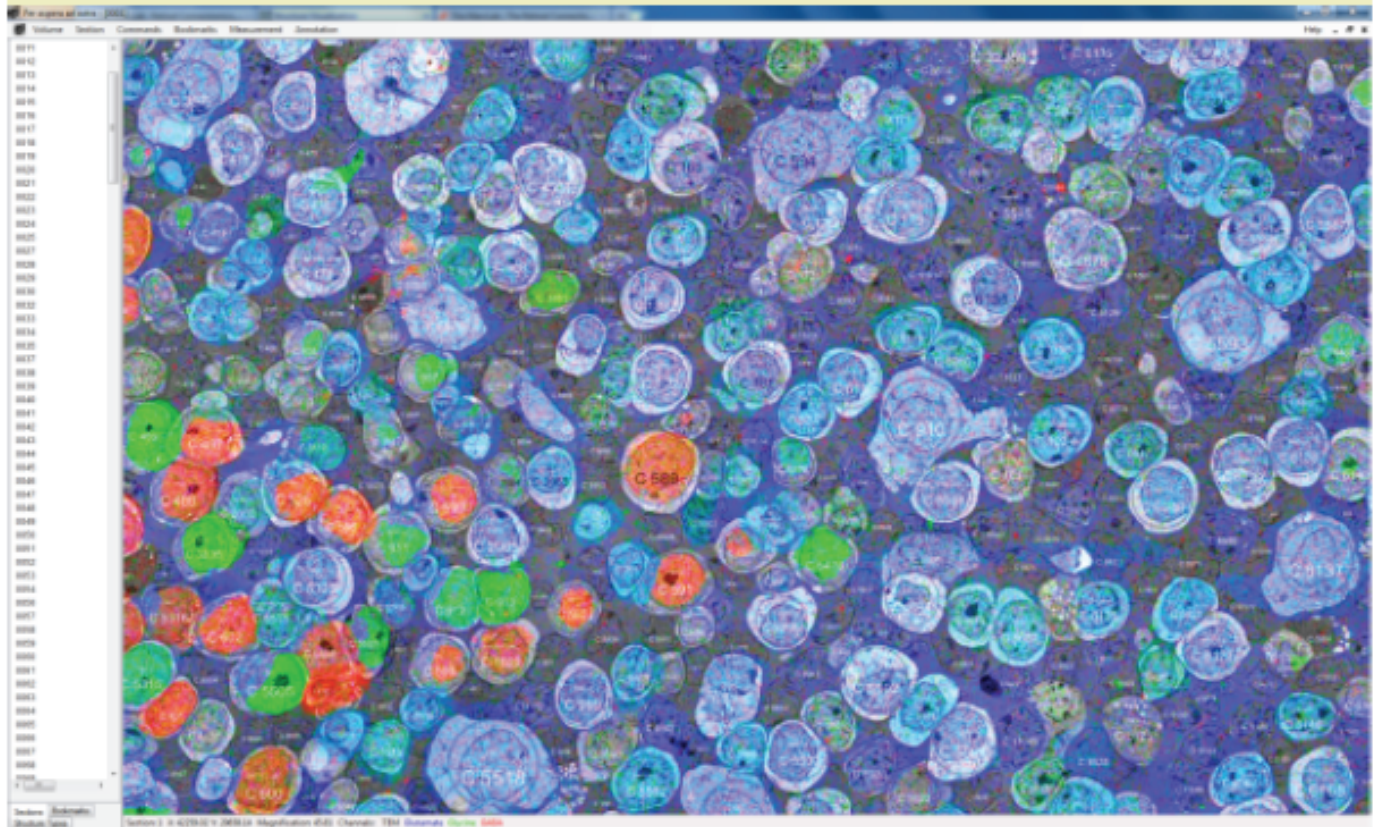# CONNECTOMICS ANNOTATION FEST 2012
## VIKING, VIZ & VIKINGPLOT

© MARCLAB 2012

Viking, Viz and **Vikingplot** are a set of tools designed to visualize, annotate and analyze connectomics datasets called *volumes*. This brochure describes how to launch and interactively use them.

Viking visualizes terabyte-scale volumes of tiled image stacks and allows detailed annotation of those volumes. via a remote connectomics server. Viking is implemented as a standalone Windows-compliant browser.

Viz is a set of network and structure visualization tools that read volume annotations. Viz is implemented as a web-service through Firefox, Chrome or Safari.

Vikingplot is a 3D rendering tool that captures Viking annotations and allows independent display of cells or groups of cells. It is a Matlab application called via command line.
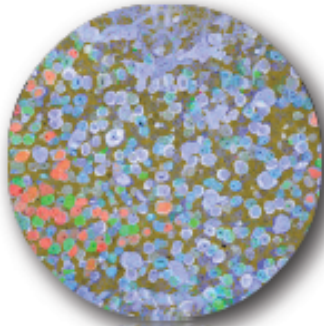
LAUNCHING VIKING

Viking uses a Microsoft environment for images, data and connectivity. A PC must have properly configured network access and some additional system resources may needed.

See the **Viking Installation** page.

Viking can be operated in a read-only mode that only requires an Anonymous Login or in a full annotation mode. The latter requires an authenticated username and password.

Viking can select from a library of volumes. The features enabled in Viking depend in part on the volume being explored.

LAUNCHING VIKING

Launch Viking from the Start Menu, desktop or toolbar
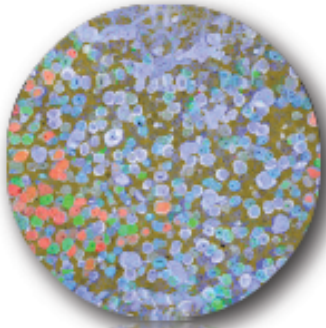
The client will contact the connectomics server

The login will request your credentials

You may select a volume to view
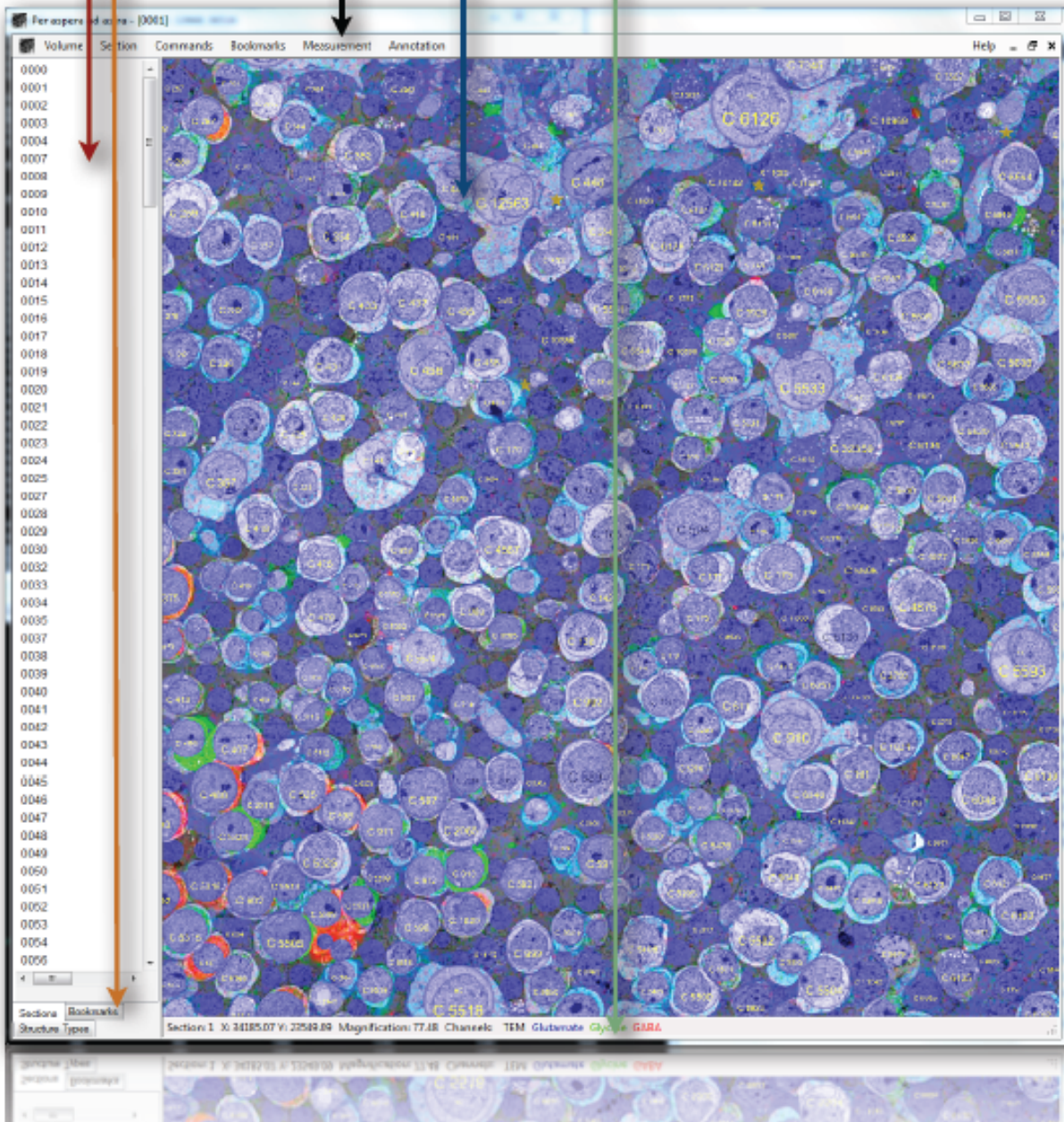
The images and transforms will load
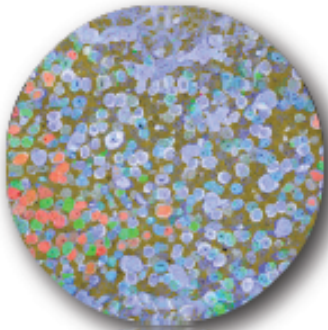
VIKING INTERFACE

VIKING COMPONENTS

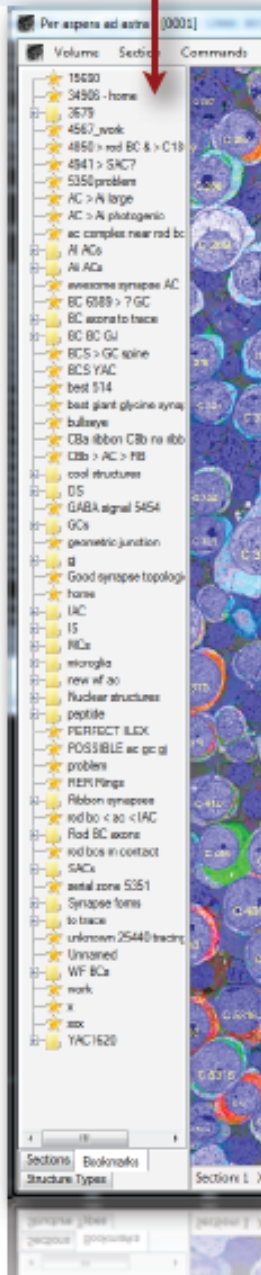**Tab Window & Tabs**   **Menu**   **Viewer**   **Status Bar**
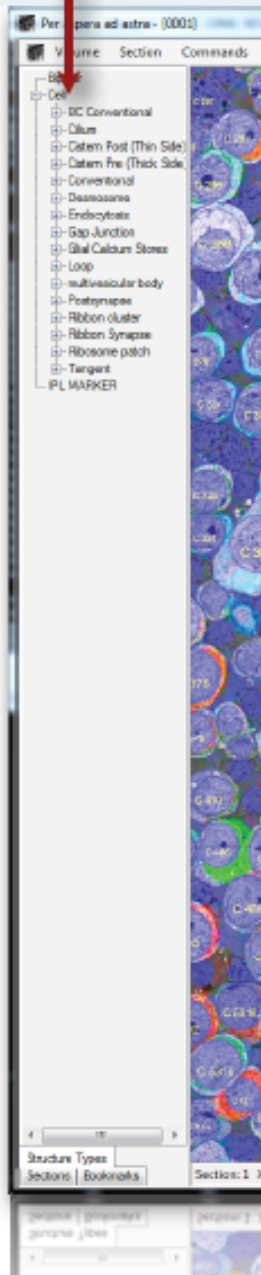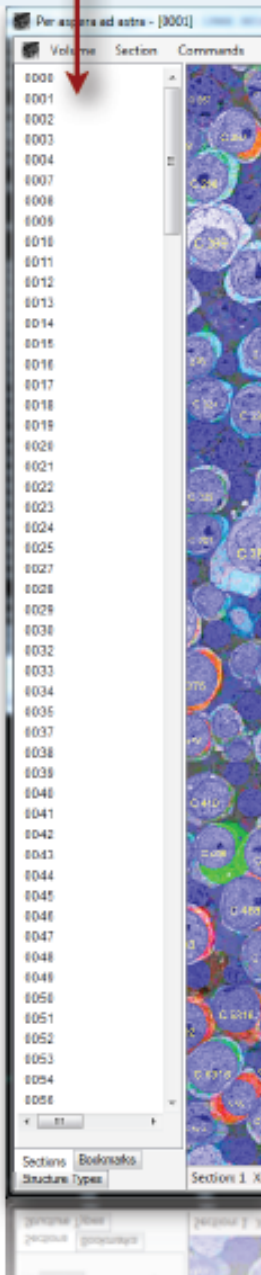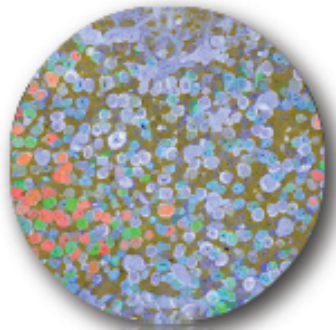
VIKING INTERFACE
Tab Window & Tabs

Right click in any tab opens a specialized
dialogue box

Sections displays the ordinal slice layout

Structure Types displays annotation choices

Bookmarks displays personal annotations

VIKING INTERFACE
Menu

Choices useful for annotation are in
gold. Others are greyed.

Volume

Transform → None	Displays the untransformed slice
Transform → grid16	Displays the volume transformed slice

Section → Channel ...
Section → Section specific transforms
Section → Show mesh
Section → Setup channels
Section → Colorize tiles

Commands

Go to location	Jumps to location XYZ. Ctrl v pastes the coordinates
Capture screen	Opens dialog & cursor to select and save a Viewer ROI
Export frames	
Export tiles	

Bookmarks

Hide / Show Bookmarks	Hides / Shows the selected bookmark
Undo Bookmark change	Reverses the last bookmark action

Measurement

Set Scale	Allows user calibration. Each volume has a default scale
Measure Line	Active distance measurement

Annotation

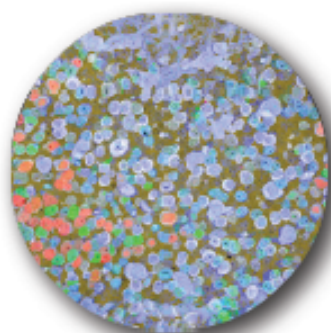|	
Open last modified location	Jumps to last good annotation
Open Structure	Opens property box for a structure
Merge Structures	Annotation correction (executive users only)

VIKING INTERFACE
Viewer & Status Bar

The **Viewer** window displays multiple kinds of data in each volume

- Greyscale image texture
- RGB image molecular channels
- Blue discs with identifying text - positional annotations
- Arrowheads indicate the location of annotations in slices above and below
- Yellow / green lines are links among parent shape annotations
- Other colored discs are child structures (synapses etc.) belonging to a parent structure (cell)
- Animated white bands are directional link between structures (synapses etc.)
- The *space bar* toggles the annotations on and off
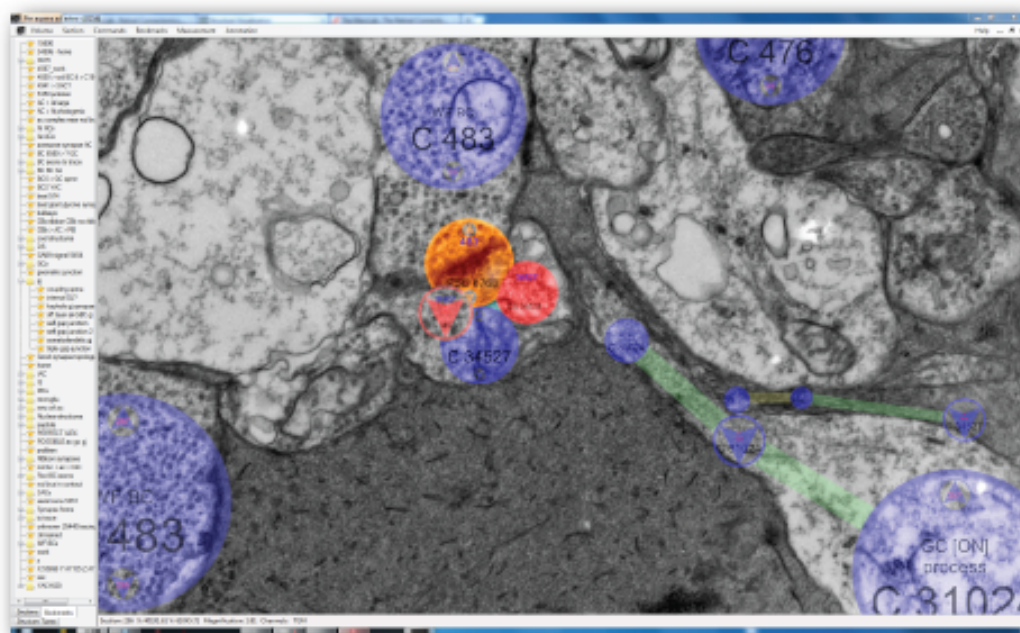
The **Status Bar** displays navigational data

- Section: slice number
- X: X location in physical pixels at magnification 1
- Y: Y location in physical pixels at magnification 1
- Magnification: actually the zoom out. Mag 1=maximum resolution Mag 10= 1/10 resolution
- Channels: TEM or molecular

Section: 1 X: 42259.02 Y: 20658.14 Magnification: 45.81 Channels: TEM Glutamate Glycine GABA
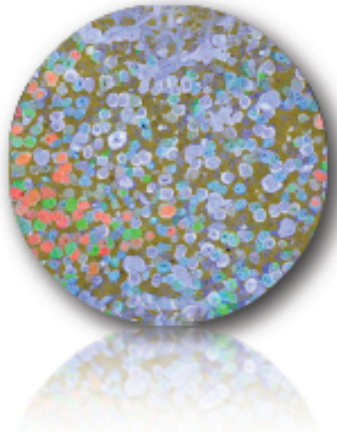
VIKING NAVIGATION

Moving around in XYZ with the mouse

- *Right click and drag* on image (not annotations) to pan across the image. Avoid annotations.
- *Right click* on annotations to open a context menu
- *Scroll wheel* zooms
- *Left click and drag* on blue arrows places new annotations
- *Left click and release* on blank area continue the last annotation
- *Side clicks* on a 5 button mouse move down and up 1 slice

Moving around in Z using the keys

- *+ (really =) and -* move down and up 1 slice
- *SHIFT + and -* move down and up 10 slices

Moving around in XYZ with the menu

- Commands → Go to location → lets you to enter or paste (ctrl v) & jump to a site
- Ctrl c copies a location
- Annotation → Open Structure → opens a navigation dialogue for a cell
- Bookmarks can be used (see below)

Other keys

- spacebar = toggles annotations
- C = place a new cell annotation
- P = place a new postsynaptic annotation
- S = place a new conventional synapse annotation
- R = place a new ribbon synapse annotation
- D = place a new adherens junction annotation
- G = place a new gap junction annotation

WORKING WITH *Viking!*

HOW *Viking!* WORKS

Viking! is a program designed to reconstruct a 3-dimensional image of the connectome.

1. The tissue is isolated and processed for electron microscopy (EM).
2. The processed tissue is then cut into very thin slices.
3. The slices are digitally imaged using transmission electron microscopy (TEM). To acquire high-resolution images, small areas of the field are imaged at high magnification and a montage of the entire field is created.
4. The slices are then digitally fitted together to make a complete volume.
5. *Viking!* allows cells to be reconstructed throughout the depth of the volume in order to create accurate 3D images.
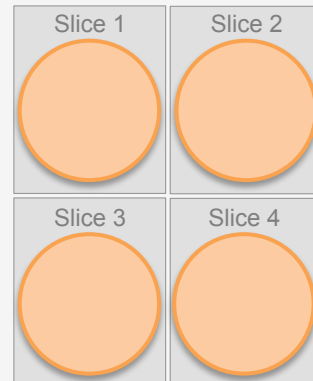
THE BASICS

What cell looks like intact

If the cell is sectioned at regular intervals…
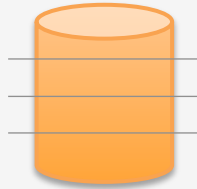
Section 1
Section 2
Section 3
Section 4

Slices will look like this when viewed from the top

Slice 1   Slice 2

Slice 3   Slice 4

Viking! allows the user to see all of the cells in a given volume of tissue. Keep in mind that orientation in 3-dimensional space is highly variable, so the portions of cells visible as images in *Viking!* will have many different shapes.
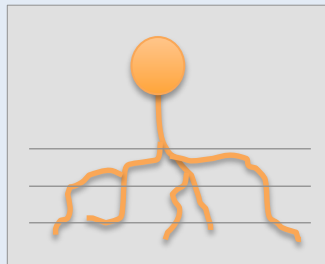
WORKING WITH *Viking!*
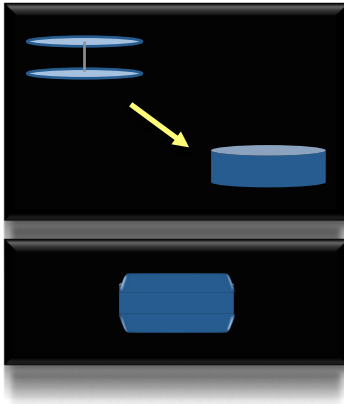
HOW VikingPlot RENDERS ANNOTATIONS

Viking! connects the edges of the 2-dimensional discs the user places onto cells in order to reconstruct a 3-dimensional representation of the cell

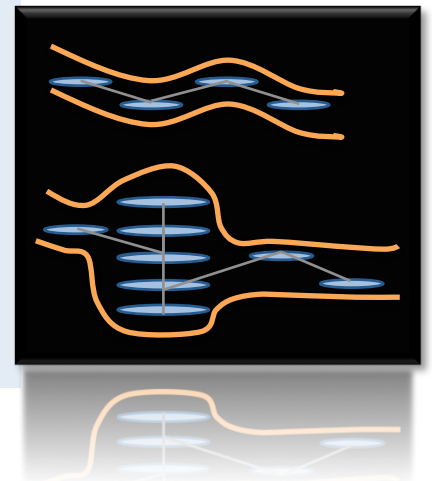*Viking!* then puts rounded caps on the top and bottom discs to make a more realistic, rounded shape

Note: *Viking!* prefers that discs for a given cell be placed on alternating slices.
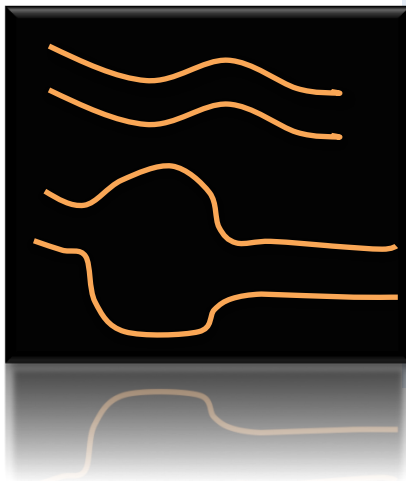
ANNOTATION APPROACHES

Use:
"Hopscotch"   to illustrate   longitudinal cells

Use:
"Stack of Coins"   to illustrate   varicosities, etc.

ARROWHEADS

Viking! creates arrowheads automatically.
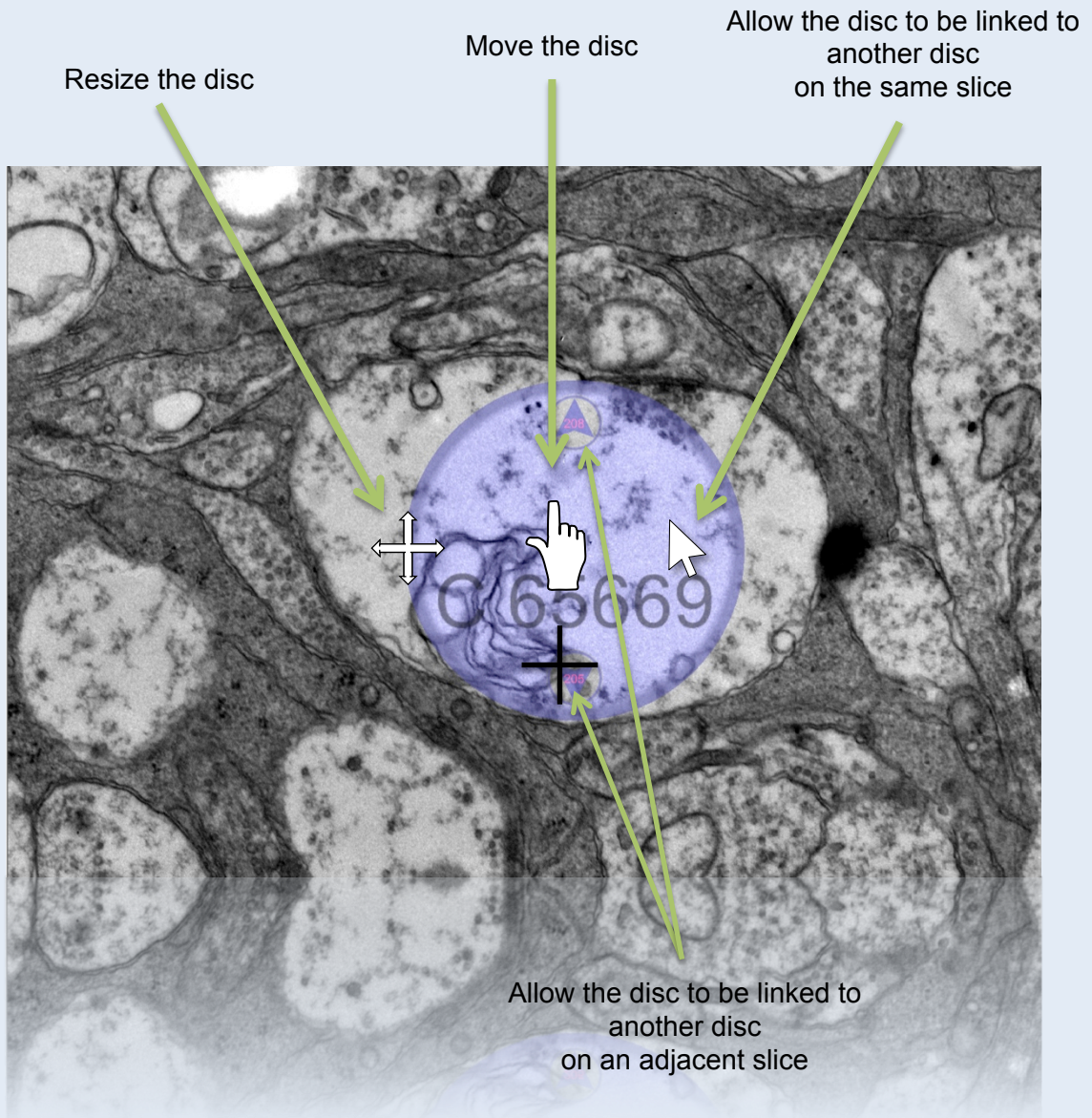
Arrowheads simply indicate that an annotation for the same cell, synapse, etc. exists on a section immediately adjacent to the one being viewed.
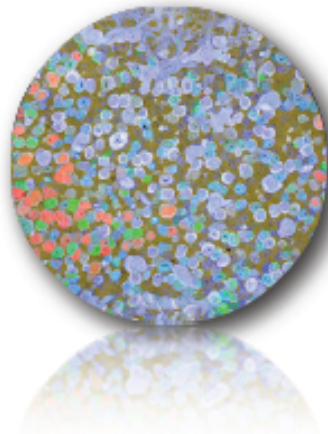

XXXX

WORKING WITH *Viking!*

CURSOR POSITIONS WITHIN DISCS

Placing the cursor over different locations within a disc will (cursor icons enlarged for clarity):

Resize the disc

Move the disc

Allow the disc to be linked to another disc
on the same slice

Allow the disc to be linked to
another disc
on an adjacent slice

Creating a cell :: A cell can be created at any locus and traced

1. **Start.** Place your cursor in the Viewer. Hit the "c" key. A large blue C will appear. Move to and click on the target cell, drag and release to place the first disc in the cell. Double right click the blue disc, open its PropertySheetForm and give it a Label (see the Approved Label List later in this document).

2. **Link to a slice.** Page to the next slice (+ or -), left click on the cell and drag the next disc. It will be automatically linked to the previous disc. Repeat 2 as often as desired. Note that the text in the disc will be yellow to indicate that it is the last active disc in a series. When the next disc is added, a yellow one will become black. Note that there is small blue arrow on the adjacent slices to indicate the location of the previous disc.

3. **Skip a slice.** Some slices may have defects that prevent finding your cell. Skip to the next good slice, left click and drag a disc. It will be automatically linked to the previous disc.

4. **Link on the same slice.** In general, a single contour should contain only one linked disc on a slice but there are exceptions: very large tangential processes, rapidly narrowing tangential processes and branches are the prime instances.
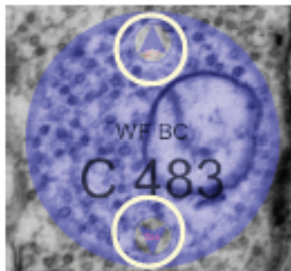

Note that every disc has two arrows in it that indicate the numbers of the next and previous slices. If you click on an arrow it will let you drag a link and create a new disc.

5. **Erase.** If you make an error you can delete one of more discs individually. Right click and select "delete."

6. **Repair.** Sometimes links may be broken and require remapping. Combined operations 5 and 6 will usually solve the problem.
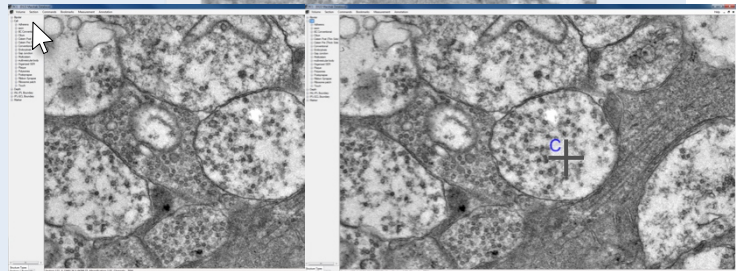
11

WORKING WITH *Viking!*

CREATING ANNOTATIONS (ILLUSTRATED)

1. Place the cursor over the feature you would like to annotate.
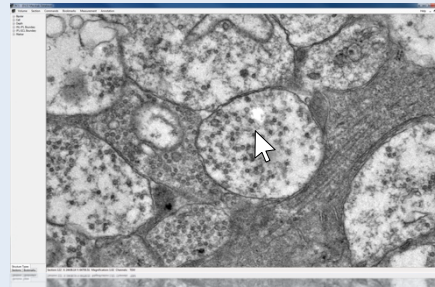
2. Press the hot key (or select it from the **Structure Types** menu on the bottom left, then left click to set the annotation) for the desired feature.


3. Drag the cursor to expand the disc to the desired diameter (or in the case of an annotation selected from the Structure Types menu, place the cursor on the edge of the annotation, left click once, and then drag to expand).
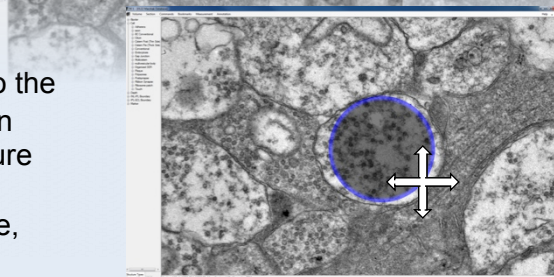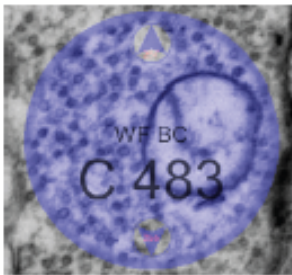

4. Left click once to set the disc.
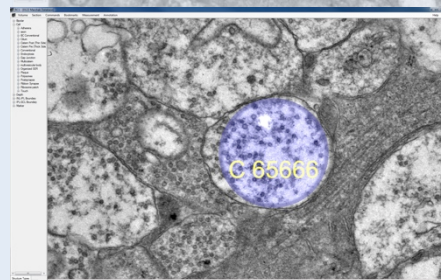
If the wrong hot key/Structure Type is selected, press the **Esc** key to cancel. If the disc has already been created, double right click to open options and then select **Delete**.
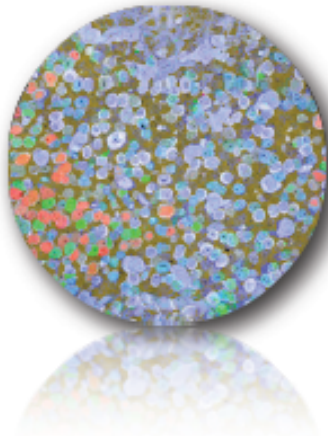
VIKING ANNOTATION
COMPONENTS

Each annotation a part of a parent structure (a cell) or a child structure (presynaptic, postsynaptic, gap junction, adherens, etc).

Parent Structures

Cell A cell is a set of linked blue discs that sculpt the cell's shape. The cell structure index (e.g. 483) is displayed on every disk along with the cell's Label (WF BC). This index the key entity in navigating Viking, Viz and Vikingplot. The Label is stored in cell's PropertySheetForm and is accessed via a double right-click or Menu → Annotation → Open Structure.
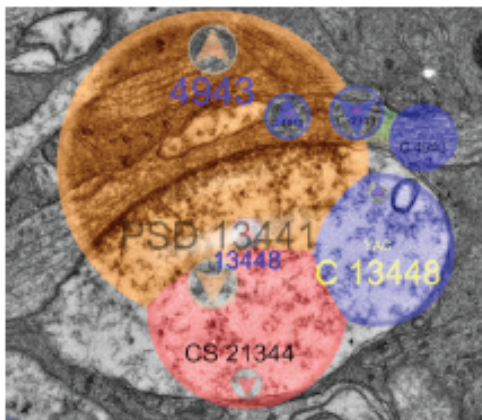
Child Structures

Child structures are sets of linked colored disks that belong to one and only one cell. However sets of certain child structures like synapses are connected to partners in other cells. In the example a left there are two cells: YAC 13448 and YAC [AI] 4943.

- YAC 13448 is the parent of child structure CS 21344
- YAC [AI] 4943 is the parent of child structure PSD 13441
- CS 21344 and PSD 13441 are connected (radar bands)

Current Child Structures (** paired)

- BCS = BC Conventional **
- Cil = Cilium
- Cistern Post = CPst **
- Cistern Pre = CPre **
- CS = conventional synapse **
- D = desmosome **
- Endo = endocytosis
- G = gap junction **

- RibCl = ribbon cluster
- R = ribbon synapse **
- Y = Tangent **
- PSD = postsynapse **

- OSER = Organized SER
- L = Loop
- MVB = multivesicular body
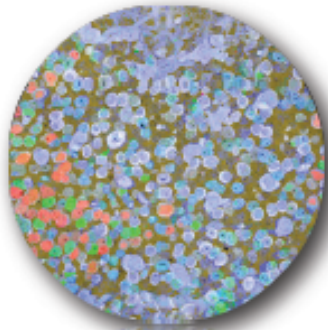
VIKING ANNOTATION
CHILD ACTIONS

Creating a child :: A child can be created at any locus and traced. The example given is for a conventional synapse. Note that standalone child structures are not allowed. Synapses are bipartite structures that require two cells for completion. If there is only one cell, you can only create half of the synapse.

1. **Start - Postsynaptic Cell**. Place your cursor in the Viewer. Hit the "p" key. A large orange P will appear. Move to and click on the target cell, drag and release to place the first orange disc in the cell. A colored band will appear attached to your cursor. Click on the parent cell blue disc and the band will disappear. This links the child to the parent. The index of the host will appear above the index of the child PSD.

2. **Link to a slice**. Synapses often span several slices. Page to the next slice (+ or -), left click on the child and drag the next disc. It will be automatically linked to the previous child. Repeat as needed.

3. **Start - Presynaptic Cell**. Place your cursor in the Viewer. Hit the "s" key. A large red S will appear. Move to and click on the target cell, drag and release to place the first red disc in the cell. A colored band will appear attached to your cursor. Click on the parent cell blue disc and the band will disappear. This links the child to the parent. The index of the host will appear above the index of the child CS.

4. **Link to a slice**. Synapses often span several slices. Page to the next slice (+ or -), left click on the child and drag the next disc. It will be automatically linked to the previous child. Repeat as needed.

5. **Connect the synapse**. Left click in the presynaptic child CS and drag the black band to the postsynaptic child PSD. Release and animated white radar bands will appear emitting from presynaptic side.

6. **Correct the connection**. Sometimes you will drag the wrong direction or to the wrong target. Right click on the radar and you will be able to "flip" or "delete" the connection. Some bipartite structures like gap junctions and desmosomes (adherens junctions) have no implicit directionality. Right click will let you make a connection "bidirectional."

WORKING WITH *Viking!*

SUMMARY:
- Cells are **parents.**
- Synapses, gap junctions, etc. are **children** belonging to their associated parent.
- To annotate a child structure, the associated parent must be annotated.
- Each child structure must be linked to a parent.
- Annotate child structures in all slices in which they appear.

HOW TO LINK CHILD ANNOTATIONS (ILLUSTRATED)

1. If you are annotating a child structure, first add the appropriate structure disc. After setting the disc, a colored band will appear. Without clicking, move the cursor to the center of the parent's disc and left click once to link the two discs.
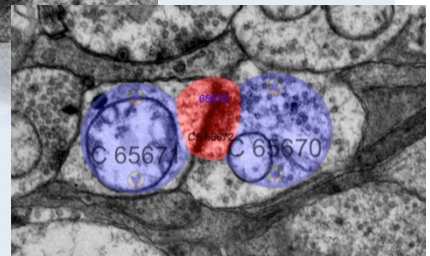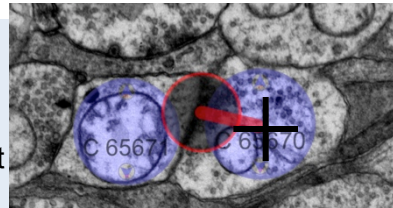

2. For synapses, adherens, gap junctions, cisterns, etc., you must specify the direction of the communication.
   - Place the cursor inside the disc of the pre- partner and left click once
   - Drag the cursor to the disc of the post- partner, then left click once
   - An animated white band will appear and move in the specified direction of the interaction.
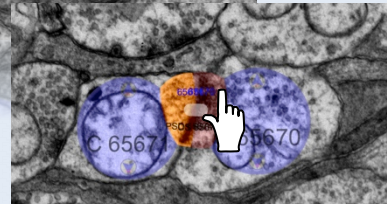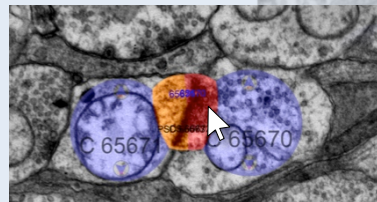
For bidirectional annotations, place the cursor between the partner discs, right-click twice, and select Bidirectional.
   - Directional: conventional, bipolar conventional, ribbon, cistern, etc.
   - Bidirectional: gap junction, adherens, etc.
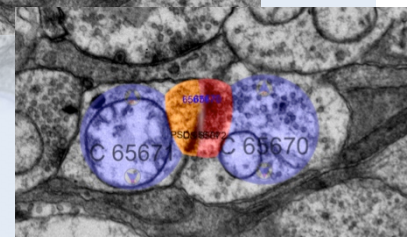
WORKING WITH *Viking!*

HOW TO ANNOTATE A BRANCHING CONTOUR

This is an exception to the preference of *Viking!* for discs to be placed on alternating sections.

Let's say, for instance, that you'd been following the primary dendrite of this ganglion cell up through the volume, and it branches between slices 3 and 2. How does one annotate this?

At this point, you know that the cell is going to branch because you've conducted at least a brief survey ahead.
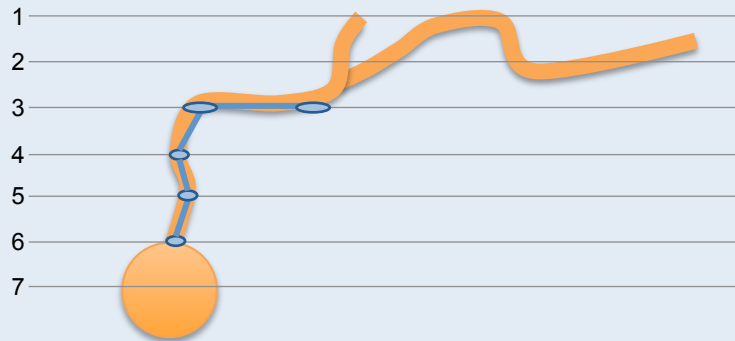
You will recall that:

1) *Viking!* uses the discs to reconstruct a 3D image of the cell; therefore, it is important to place discs with as much accuracy as possible.

2) Every disc has two arrowheads in it. If you click on an arrowhead, it will let you drag and create a new linked disc.
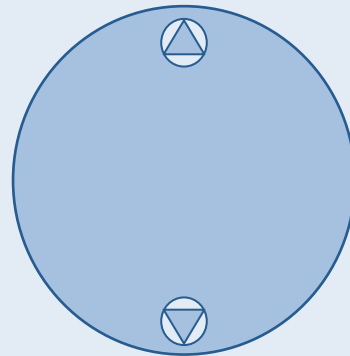
So, if you link a new disc on slice 2 to the existing one on slice 1, the rendering will look like this:

New linked disc   Rendered:
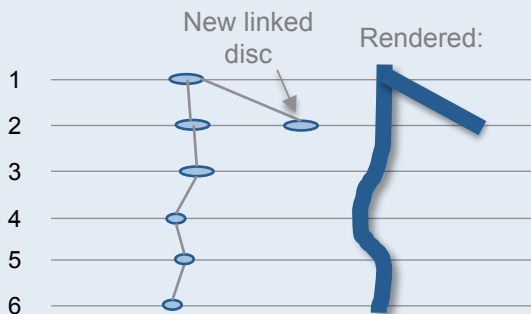
…And if instead you link it to the disc on slice 3, it will look like this:

New linked disc   Rendered:
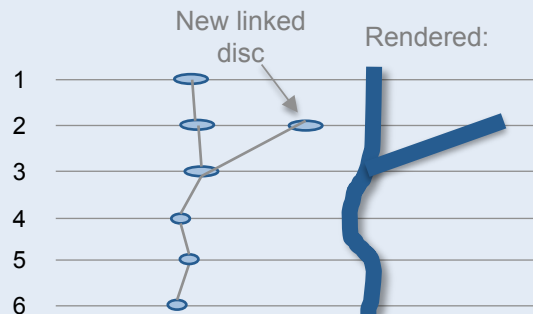
The way to decide which disc to link a new one to (in this case for a branch point) is to follow the shape of the cell within the volume.
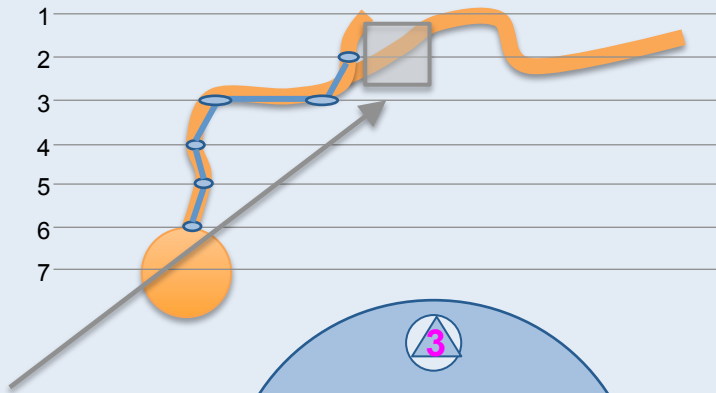
WORKING WITH *Viking!*

HOW TO ANNOTATE A BRANCHING CONTOUR

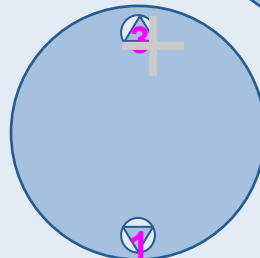First, place a linked disc the usual way on the primary dendrite in slice 2.

Next, add a linked disc onto the branch in slice 2…

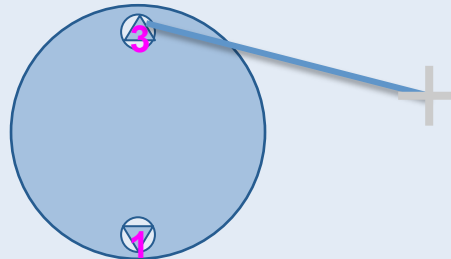In this case, you want to add a new disc onto slice 2 and link it to the one on slice 3.

Seen here is the disc on slice 2 from above.

1) Place the cursor (it will be a simple crosshair) over the arrowhead for the slice you want to link the new disc to (slice 3).

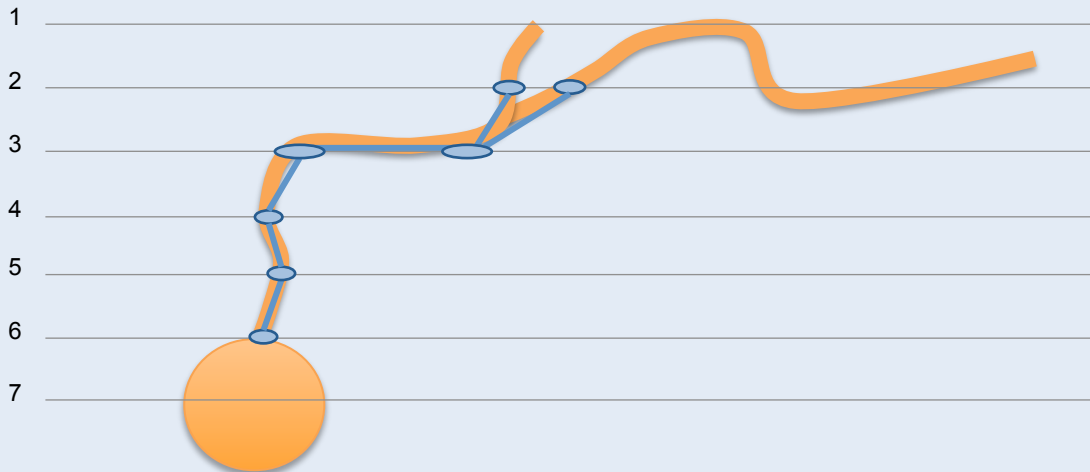2) Left click once, release, and drag the cursor to the place you want to add the new disc.

3) Left click once to set the center of the new disc, drag the cursor out until the new disc is the desired size, and left click to set.
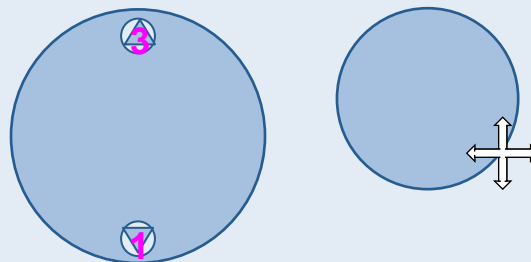
WORKING WITH *Viking!*

HOW TO ANNOTATE A BRANCHING CONTOUR

You have now generated this:

Both branches may now be annotated as usual.

This scenario highlights the need to survey a contour before annotating so that one formulates a plan for annotation and avoids surprises

ANNOTATION DISC

Annotate at the center of mass of a cell without exceeding the bounds of the plasma membrane – even for oddly-shaped or uneven cells. For example, the irregular cell seen below would be annotated as follows:
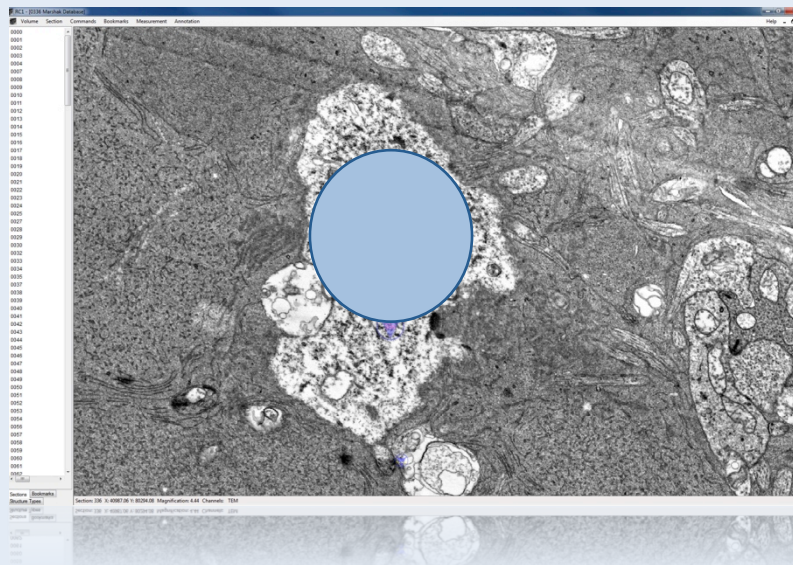
WORKING WITH *Viking!*

WHAT TO DO IF YOU SEE TWO ARROWHEADS BUT NO DISC OUTLINE

Sometimes you may see one or two arrowheads indicating the presence of a disc, but be unable to see the outline of the disc. In addition, the field may have a grey cast to it.
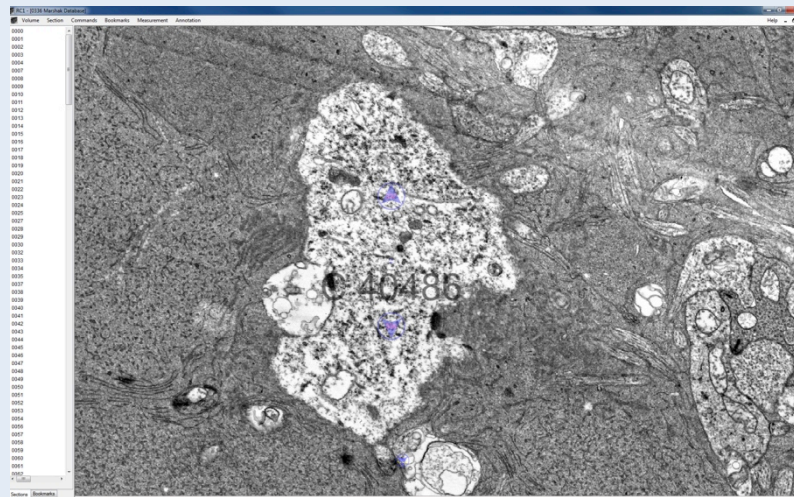
This is an indication that the disc is *very* large.

Simply zoom out and resize the disc (or delete if it was placed by mistake).

Volume field

Giant disc