IGL Trace v1.26b

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Index

Overview

IGL Trace is a program for identifying, tracing, measuring and reconstructing three-dimensional objects in a series of sections using a personal computer. Serial section images are typically obtained from electron or confocal microscopy. It is then desired to obtain the three-dimensional structure and visualize it using computer graphics. IGL Trace and related programs allow images in a series to be aligned (if necessary) and an outline of the structures within the images to be created. The outlines, referred to as contours in IGL Trace, form the basis for a three-dimensional (3D) reconstruction of the structure which can be rendered on the computer to produce a revealing visualization of the object not available in the two-dimensional (2D) section images. In addition, 2- and 3-D measurements can be obtained from the contours.

Family of Programs
System Requirements
File Organization

IGL Family of Programs

IGL Trace is designed to work in conjunction with a few other programs for a complete system for reconstruction and visualization of serial sectioned objects.

IGL Align and sEM Align are programs for alignment of section images. Alignment of sections is typically performed prior to using IGL Trace. Alignment facilitates identification and tracing in serial sections. IGL Align is an outmoded program with specific hardware requirements and limited capabilities. The newer sEM Align is much easier to use and will run on any PC. For more information consult the online documentation (http://synapses.bu.edu/) for these programs.

Another useful program is a VRML viewer. IGL Trace outputs 3D objects as VRML 1.0 files. These can be viewed using a VRML 1.0 viewer. If a stand-alone viewer is available, such as VRweb Viewer, IGL Trace can be configured to automatically invoke the viewer whenever a 3D object is created. To do this, edit the trace.ini file and enter the full path to the VRML stand-alone viewer.

Example:

```
[VRMLviewer]
Path=C:\VRweb\vrw_nt.exe
```

If a VRML 1.0 viewer is not available, VRML 1.0 files generated by IGL Trace can be converted to another 3D format using a file converter program.

Also available online are several utilities, such as a file converter for putting a set of image files into an IGL Trace series,and a program for merging several VRML 1.0 objects into a single scene.

System Requirements

IGL Trace can be used on any personal computer (PC) capable of executing Microsoft Windows(tm) Win32 programs. This includes Windows 3.x running Win32s, Windows NT, 95, 98, and 2000. The software was developed and tested under Windows NT 4.0.

The system should have a video graphics adaptor configured for at least 800x600 pixels and 4,096 colors. This is needed to adequately display the application window and the 8-bit grayscale images on which IGL Trace operates. Although IGL Trace can run on any 32-bit CPU, such as Intel 80386, 486, or Pentium processors, hard drive speed and RAM will significantly affect the speed with which IGL Trace can page through image files. With large image files (>2Mb per section) a high performance hard drive and lots of memory are recommended. A standard workstation with a SCSI hard drive and 256Mb of RAM gives excellence performance on 10Mb image files.

File Organization

IGL Trace operates on data files which are organized into studies. A *study* is a set of serial sections given a unique name, typically stored in a single directory. Serial sections in a study are numbered sequentially beginning with values greater than zero. Section zero is reserved for a calibration image, on which quantitative spatial measurements of the data can be based. Thus, section image data is designated by a study name and an integer section number.

Each section has a set of contours associated with it. The files containing contour data reside in the same directory as the image files, and are denoted by the study name and the section number.

The following lists the different file names used by IGL Trace for a hypothetical study named ‘hippo’. Section files always end in a number separated from the study name by a period.

- Image files: _hippo.0, _hippo.1, _hippo.2, ...
- Contour files: hippo.0, hippo.1, hippo.2, ...
- Calibration file: hippo.clb
- Backup files: ~hippo.1, ~hippo.2, ...
- Various export files: hippo.wrl, hippo.cnt, hippo.igl, hippo.map, etc.

IGL Trace accepts digitized section images in a variety of image formats. (For a list of acceptable formats, access the Input Formats menu item from the Help menu of IGL Trace.) Whatever the image format, the file naming convention described above must be adhered to. Thus, .bmp files must be renamed to ‘_hippo.1’, etc. Files with different names can be imported into the IGL naming convention using the separate file converter or the Import command from the Section Menu.

Although IGL Trace accepts color images, a number of the image filtering and automated tracing tools operate only on 8-bit grayscale intensity images, so IGL Trace provides facilities for conversion to the proprietary IGL image file format. When section images are saved in IGL Trace, an attempt is made to maintain the initial format if it is Windows Bitmap or IGL, but if a format cannot be maintained, e.g. when a user requests the image file have an annotation, the image is always converted to the IGL format. Images files may be exported to Windows Bitmap format. Contour files also use a proprietary format but allow exporting to other contour file formats.

For details on file formats used by IGL Trace see the IGL Trace File Formats topic.

User Interface

The user interface presented by IGL Trace consists of the application window which contains a set of command menus at the top for user selection of various operations, a status bar at the bottom for display of command help messages and contour information, a image display area for display of section images and tracing of contours, and a palette or toolbar of edit tool buttons which can be selected with the mouse. There are number of other functions which can be carried out by special key strokes called accelerators. These include deleting contours or undeleting them, zooming in and out, backing up when drawing, and paging through the study. Efficient use of IGL Trace requires that the user become familiar with these accelerators.

Application Window
Menus
Toolbar
Accelerators

Application Window

The IGL Trace application window consists of a frame with a caption bar at the top, a set of top-level menu items beneath the caption bar, and a section display area below this. To the left of the section display area is a tool box of tool buttons. At the bottom of the application window is a status bar. The window will be described as it appears in Windows NT. It may appear slightly different in other operating systems.

The IGL Trace window can be moved by dragging the caption bar with the mouse. Alternatively, the window can be moved by clicking the bar icon to the left of the caption, selecting the *Move* item from the drop-down menu, and then dragging the window with the mouse. The window can be minimized by either selecting *Minimize* from this drop-down menu, or clicking on the down arrow icon to the right of the caption bar. To reopen a minimized IGL Trace window, locate the IGL Trace icon at the bottom of the display and double-click on it. The left drop-down menu can be used to exit IGL Trace by selecting *Close*.

The application window can be resized by moving the cursor to a side or corner of the frame, depressing and holding the left mouse button, and then dragging the frame to a new size. The up arrow in the upper right corner of the frame resizes the application window to fill the whole screen, i.e. maximizes the window. If the Fit Image to Window item is selected in the Window Menu the image will be automatically resized to fit the window. Rescaling the image to something other than its actual size may result in distortions of the image and contours displayed on the screen. To see the actual image at its actual size, use the Fit Window To Image command. This command may require you first unmaximize your window or Fit Image To Window before it will work properly.

The caption bar displays the study name and number of the section that is currently open. The status bar at the bottom has two modes of display. Normally it displays the name, length, and enclosed area of the contour under the cursor. If the length and area fields are 0, then the study most likely has not been calibrated. If a contour has a non-zero length but 0 area, then this contour is an open contour for which enclosed area is undefined. If a contour shows a negative enclosed area then the contour is counterclockwise. Only clockwise contours have a positive area. When the user presses the left mouse button on a menu item or tool button, the status bar displays a brief description of the command being selected.

The top-level menu items appearing below the caption bar can be selected with the mouse, or may be invoked by hitting the ALT key followed by the underlined letter in the menu name. For example, the Study Menu can be invoked by ALT then T. Menu items can then be selected by hitting their underlined letters. A study must be opened using either Open or New before the Section, Contours, and Window Menus will be enabled. When IGL Trace is opened the section display area initially contains a gray 512x512 area. To see a section image here, open a study. The program will automatically load and display the first section of the study.

Menus

At the top of the window, below the caption bar, there are a series of command menus. These menus use the usual Windows conventions. The underscored letter in a menu item indicates the key stroke that may be used to select that item. Use the Alt key to enter menu select mode, then hit the desired letter. For example, “t” will enter the Study menu. Keep entering key strokes to select additional subitems or escape to exit the menu.

An ellipsis (...) after a menu item indicates that the further input from the user will be required for the completion of this command. Usually these items respond with one or more dialog windows after selection. A menu item without ellipsis performs its function immediately, without further input from the user (or an opportunity to cancel the command). A right-pointing arrow after a menu item indicates that this item will popup a submenu with additional choices.

Study Menu
Section Menu
Contours Menu
3D Menu
Window Menu
Help Menu

Study Menu

This menu provides commands for entire series of sections called studies. A study can be opened, closed, created, calibrated. Opening or creating a study enables the other command menus from which actions on individual sections can be taken.

New : Create a new study.
Open : Open a previously created study.
Close : Close the open study.
Import : Import a file containing a series of section images.
Calibration : Examine or modify calibration data.
Quit : Exit IGL Trace.

Section Menu

The section menu contains operations on serial section image data. These operations include the selection of the currently viewed section among other things.

Hide : Hide section images.

Show : Display section images when hidden.

Last Zoom : Go to last zoomed view.

Zoom Out : Return to view prior to last zoom.

Goto : Go to a particular section in the study.

Previous : Go to the previous section visited.

Predecessor : Go to the next lower section number.

Successor : Go to the next higher section number.

Contrast : Open/close the contrast dialog.

Image Filtering : Image processing command submenu.

Import : Import an image into a numbered IGL section.

Save Image : Save image in IGL annotated format.

Annotation : Display and/or modify image annotation.

Export Image As : Save image in a non-IGL format.

Contours Menu

The Contours Menu contains commands for creating and manipulating contours, and for extracting information from contours.

- Hide : Do not display contours.
  - Show : Display contours when hidden.
- Delete : Delete the selected contour.
- Insert : Insert the last set of deleted contours into the current section.
- Save : Save current contour set to disk.
- Import : Import an ASCII file with contours into the current section.
- Copy all : Copy contours from another section.
- Replace with : Replace contours by placing new contours at centroids.
- Rename : Rename contours with a particular name.
- Delete all : Remove contours with a particular name.
- Find : Locate a contour by name.
- Clockwise : Reorient contours with a particular name.
- Trace Regions : Automatically trace boundaries of all distinct regions.
- Configure
  - Name Table : Open/close contour name accelerator table.
  - Wildfires : Set options for wildfire contours.
  - Color : Create a new color for default color.
- Export As : Save using an external contour file format.
- Quantify 2D : 2D information about contours.

3D Menu

The 3D menu contains operations for 3D reconstruction and quantification of the contours in a study. The recontruction items (Points, Lines, Planes, Slabs, or Surfaces) generate .wrl files which can be viewed by auxiliary programs capable of reading and displaying VRML 1.0 files. After creating the file, an attempt will be made by the program to display the file using the current VRML viewer program. The path to this program is set in the trace.ini file which you can edit with notepad. The viewer should be capable of taking the .wrl file as a command line parameter. If not, you will need to invoke the viewer outside of the IGL Trace and load the .wrl file from within the viewer.

Points : Generate a a 3D representation of the centoids of contours.
Lines : Generate a representation of 3D spatial arrangement of contours.
Planes : Generate a representation of contours as filled regions in space.
Slabs : Generate a representation of contour regions as thick slabs in space.
Surfaces : Generate a representation of the surface of the 3D object.
Quantify 3D : 3D information about objects.

Window Menu

Copy Image to Clipboard : Copy current window display to clipboard.
Print : Print current window display.
Fit Image to Window : Scale section data to fill window.
Fit Window to Image : Resize window to image actual size.

Help Menu

Using Help : How to use help and make annotations.
Using Trace : Open this help file.
Image Info : Display image format information for the current section.
Input Formats : Display list of acceptable image file formats.
About : Display version and copyright information.

Toolbar

The buttons in the tool box at the left of the application window provide functions for contour drawing which the user can activate by clicking on the tool icon. Some tools are retained after activation. In these cases, the currently active tool button is depressed for drawing tools and X'ed for color tools. When a drawing tool is active, the cursor is changed to the tool's cursor. For example, clicking on the pencil icon will activate the pencil contour tracing tool. The pencil button will be highlighted and the cursor will become a pencil.

An active tool is deselected by a selecting a new tool. If the new tool is capable of retaining activation, then the new tool becomes the currently active tool. However, some tools perform a single function, then return to the previously active tool. For example, selecting Delete while the select tool is active will delete the currently selected contour and then return to the select tool.

When a tool is deselected its present operation is terminated. For example, deselecting the pencil tool while tracing a contour will discontinue tracing of that contour. If the pencil tool is reselected while the contour is highlighted, the contour can continue to be traced. An active tool can be automatically reselected by clicking the right mouse button. In the case of the pencil tool this has no effect since the contour remains active, but in the case of the measure tool it ends one measurement and begins another without having to return to the tool bar.

Select Tool : Use to select contours.
Zoom Tool : Select and magnify region.
Pencil Tool : Draw a new contour.
Ellipse Tool : Make an elliptical contour.
Point Tool : Make a point contour.
Line Tool : Draw a contour that has a single line segment.
Split Tool : Break a contour at a particular point.
Join Tool : Connect two contour head to tail.
Expand: Expand a contour by one pixel.
Shrink: Shrink a contour by one pixel.
Wildfire Tool : Automatically trace light objects with dark borders.
Measure Tool : Make a length measurement.
Name : Name the selected contour.
Clear : Refresh the display and clear any extraneous traces, (e.g. left by the Measure Tool).
Close : Make the selected contour a closed contour. This operation is also realized by the backslash key.
Reorient : Make the selected contour clockwise if counterclockwise, or vice versa.
Colors : Select the contour color.

Accelerator Keys

Accelerator keys allow a set of key strokes to serve in place of more complicated actions. Menu items can be selected using key strokes by first hitting the Alt key and then entering the underlined letter to activate the menu item. Other accelerators for IGL Trace are:

/	Return to previous section
\	Close the selected contour
Spacebar	Save the contours for the current section
Page Up	Go to next higher section
Page Down	Go to next lower section
Delete	Delete the selected contour
Insert	Undelete the last contour(s)
Home	Go to last zoomed region
End	Return to region before last zoom
Backspace	Erase last point of selected contour
Up Arrow	Move selected contour up
Left Arrow	Move selected contour left
Down Arrow	Move selected contour down
Right Arrow	Move selected contour right
Control-Up Arrow	Move selected contour up faster
Control-Left Arrow	Move selected contour left faster
Control-Down Arrow	Move selected contour down faster
Control-Right Arrow	Move selected contour right faster
Control-Left Mouse Button	Select the nearest contour without releasing current tool
Control-Right Mouse Button	Release the selected contour without releasing current tool.
Control-0	User-definable name accelerator
Control-1	User-definable name accelerator
Control-2	User-definable name accelerator
Control-3	User-definable name accelerator
Control-4	User-definable name accelerator
Control-5	User-definable name accelerator
Control-6	User-definable name accelerator
Control-7	User-definable name accelerator
Control-8	User-definable name accelerator
Control-9	User-definable name accelerator
Control-a	User-definable name accelerator
Control-b	User-definable name accelerator
Control-c	User-definable name accelerator
Control-d	User-definable name accelerator
Control-e	User-definable name accelerator
Control-f	User-definable name accelerator
Control-g	User-definable name accelerator
Control-h	User-definable name accelerator

Control-i	User-definable name accelerator
Control-j	User-definable name accelerator
Control-k	User-definable name accelerator
Control-l	User-definable name accelerator
Control-m	User-definable name accelerator
Control-n	User-definable name accelerator
Control-o	User-definable name accelerator
Control-p	User-definable name accelerator
Control-q	User-definable name accelerator
Control-r	User-definable name accelerator
Control-s	User-definable name accelerator
Control-t	User-definable name accelerator
Control-u	User-definable name accelerator
Control-v	User-definable name accelerator
Control-w	User-definable name accelerator
Control-x	User-definable name accelerator
Control-y	User-definable name accelerator
Control-z	User-definable name accelerator

Acquiring Images

A typical study might begin with the acquisition of serial section data by photographic imaging from the electron microscope (EM). The EM negatives are then either scanned with a large format film scanner or printed and digitized with an ordinary desktop scanner. Serial section photographs are organized into their proper sequence, beginning with a photograph of a calibration grid magnified and reproduced at the same exact scale as the remaining sections.

In IGL Trace, a new study is created by selecting New from the Study Menu. The user is then prompted for the desired name of the study. Prior to beginning a new study, the user should establish a disk directory for storage of study files. Make sure that the device being used for file storage has sufficient space to hold the study. A typical study of say 30 512x512 serial section images would require about 8Mb of disk space.

Digitized images can be aligned using the related program sEM Align. Aligned images are then brought into IGL Trace for tracing and reconstruction. If alignment is not necessary, images in a variety of formats may be setup as numbered sections in IGL Trace by using the Import function in the Section menu. Review the basics of the File Organization to get a better understanding of section numbering in IGL Trace.

Series of images acquired from the Noran OZ confocal microscope can be brought into IGL Trace by the Import command in the Study menu. Such images are already aligned and in sequence. IGL Trace automatically separates the InterVision data file into sequentially numbered sections and generates a calibration file based on the InterVision header data.

Calibration

Acquire an image of the calibration grid at the same magnification as the serial section images. When using a scanner, make sure that the scanner dpi parameter is the same for all section images and calibration grid. Save calibration grid image as section number zero. IGL Trace expects to find the calibration contours in section zero.

The next step in performing a calibration is to draw some calibration contours on the image. This is done using the regular drawing tools. First, select Goto from the Section Menu. Section number zero should be available in the dialog box. If it is not, make sure you are in the correct directory and have properly acquired and saved the calibration image as described above. Double-click on section zero in the section list box to display the calibration image in the application window. Select the Line Tool from the tool bar. The cursor will become a line with crosshairs.

To draw a calibration line, first determine two points in the calibration image a known distance apart. Move the crosshairs to the first point. Click and hold the left mouse button. Then move the crosshairs to the second point. Release the left mouse button. A straight line contour will be drawn between the calibration points. Do not close the contour. Calibration contours are open contours.

At least two calibration contours are required. One in the y- (or vertical) direction, and one in the x- (or horizontal) direction. Give each calibration contour a unique name, such as ‘xcal1’, ‘xcal2’, ‘ycal1’, ‘ycal2’, etc. so that it will be clear which length go with which contour.

To complete the calibration procedure, select Calibration from the Section Menu. This menu item will lead you through a series of questions in which you enter the lengths of the calibration contours and the section thickness of the study. This data can be modified at any time by reselecting this menu item.

The calibration procedure compute scale factors in microns/pixel for both the x- and y-directions. This allows the contours traced in pixel coordinates on the images to be converted to micron units. Scale factors are computed by minimizing the error between the user’s input lengths and the scaled pixel lengths of the calibration lines. Thus, more lines should give a more accurate calibration. But in the case of more than one calibration line in each direction, the values stored for the calibation lines will necessarily be different from the user’s input, since all lines contribute to the computed length.

Tracing and Naming

To identify and delineate objects in a study, contours which outline the objects must be traced on the images and given names to identify them through the series.

Tracing is performed with the aid of the tools in the Toolbar. To trace a contour with the Pencil Tool, first Goto a section in which the object to be traced is visible. Select Name from the Toolbar and enter a name for the object. Contour names are limited to a maximum of 8 characters. Contour names serve to distinguish objects and relate contours between sections. Contours with the same name in adjacent sections are assumed to part of the a single physical object. For this reason, contour names should be unique within a section, unless the objects do not extend beyond the section or the contours are part of the same object. Likewise, contours which are part of the same object within a section or in adjacent sections should be given the same name.

Select the Pencil Tool from the tool bar and move the pencil to the point on the section image in which the trace will begin. Press the left mouse button to start the contour. Move the mouse to the next desired point in the contour and click the left mouse button again. Continue adding contour points around the object perimeter. When the contour is nearly complete, close it by hitting the back slash key on the keyboard. This will end tracing of the contour and will connect the end point to the start point.

Contours should be free of loops and self-crossings. For example, a figure “8” is not a valid closed contour because its interior and exterior are not clearly defined by the orientation of the contour. Often it is more reliable to draw contours while zoomed in to the region of interest, since this guards against loops produced by inadvertant mouse movements. Closed contours should be drawn clockwise around the interior of the object to clearly define which part of the object is interior and which is exterior. This is especially important for distinguishing holes from exterior boundaries. A hole would be counterclockwise, while the exterior boundary would be clockwise, even though both are a boundary of the object and therefore have the same contour name.

Select a Contour

An individual contour can be selected for further processing by using the Select Tool. Alternatively, a contour can be selected by clicking the left mouse button while holding down the control key. This method allows a contour to be selected while using any tool.

Only one contour can be selected at a time. The selected contour is highlighted as a dashed line. Once a contour is selected it can be subjected to a variety of operations. These include deletion, closing, opening, naming, changing the color, using it as the start of a wildfire, reversing its orientation, and getting 2D or 3D information about it. A contour can be unselected by holding the control key down and clicking the right mouse button.

Eliminate Extraneous Contours

Sometimes contours are unintentionally placed into a section, with the pencil tool, for example, or by using Trace Regions. Contours can be deleted individually by selecting each one with the cursor and using the delete button. A mistakenly deleted contour can be recovered immediately by hitting the Insert key. Once another deletion has been performed however, the original deletion is permanently lost (except by not saving the contours.) Only the last deletion operation is remembered.

It may be necessary to find the extraneous contours so they can be deleted. One method for locating contours in a section is to use the Select Tool over the section image. Make sure that the contours are being displayed by selecting Show from the Contours Menu if it is available. Now select the tool button that resembles the arrow cursor. This is the Select Tool. Move the cursor over the section while watching the status bar display. The status bar displays the name of the contour that is nearest the cursor. When an extraneous contour is located, click the left mouse button. This will select the contour. The contour will be highlighted and the status bar will continuously display the contour name, even when cursor position moves away from the contour. To delete the selected contour, click on the Delete button in the edit tool bar or hit the Delete key on the keyboard.

When it is difficult to find the contour to be deleted by scanning with the cursor, try using the Find operation in the Contours Menu. Find locates the first occurrence of a particular named contour in the current section and selects it. Once the contour is selected, it can be deleted as described above. More directly, the Delete command from the Contours Menu can be used to delete contours, but this will delete ALL contours with a given name. To prevent the deletion of desired contours, these should be renamed prior to selecting the Delete menu item.

Repeat a Contour Shape

Occasionally a section contains many objects of the same size and shape that are to be identified and traced. IGL Trace contains tools designed to help eliminate the tedium of this task. The basic idea is draw the shape only once and then repeatedly stamp this shape at the appropriate locations in the image. There are two methods for marking locations for repetition, an automatic method involving correlation, and a manual method using point contours. These two methods may be used singlely or in combination to achieve the desired result.

To use the manual method, set the default contour name and color to a desired value, then select the point contour tool from the toolbar. At the center of each object, place a point contour by clicking the left mouse button. When all the contour locations have been marked, select an appropriate drawing tool and drawn the final desired contour shape of one of the objects. Give this new contour a different name from that used to mark the centers, probably the final desired name for the objects. Then use the Replace with... command from the Contours menu to replace all the contour centers with the final desired contour shape.

The automated approach uses the Correlation command from the Image Filtering menu. The success of this method depends on how well the pixels inside a template contour yield a positive response when the template region is correlated with the whole image. In other words, the template will be placed at every location in the image and a degree of match determined. A strong match will lead to a positive value in the output image which can be used to locate the centers of the repeated contours.

First, draw a template contour around an exemplar of the objects to be labeled. Preferably the object should be positive (white) with respect to background. It may be necessary to reverse the contrast or otherwise filter the image before attempting a correlation. The template contour may need to include not only the object but some of its surrounding background if this helps to distinguish the objects' characteristic shape. You may need to experiment with different templates before finding an appropriate one.

Select the Correlation command using the template contour. A new image will be presented which represents the degree of match at every image location. WARNING: Do not save this image as it will overwrite you real image data! Use the Threshold command in the Contrast dialog to highlight just the peaks of the correlation. You may need to play with the threshold to get the maximum number of peaks without admitting spurious ones.

When the peaks are located (as distinct white regions on a black background), use Trace Regions from the contours menu to automatically draw contours at these peaks. Save these contours. Reload the real image data by using Goto... from the Section menu. Now use Replace with... as in the manual method to replace these contour centers with the final desired contour shape.

Create a Montage

You can normally scan an entire micrograph at high resolution and then work on pieces of the image by zooming in on them. However, it may not always be possible to acquire an entire field at high resolution. It may only be possible to acquire subparts of the field as separate images. In this case it may be desirable to put these subparts back together to get a more complete picture of the whole field. This process is called montaging.

IGL Trace trace does not directly create a montage, but can handle large images and zoom in on subparts for tracing, counting, and measurement. A montage can be created using available image manipulation packages such as Adobe Photoshop or Corel PhotoPaint. To create a montage with these packages first collect the subfield images in a common format. These image subfields should overlap each other to avoid gaps in the montage. Open one of the images in the image manipulation package and and resize the paper to a size big enough to hold the entire montage. Then paste in each image file, translating and rotating it to align its border with that of the images already merged into the montage. When all images have been merged, save the final image in a format readable by IGL Trace.

Measurements

Trace provides on-screen display of contour length and enclosed area once a study has been calibrated. This measurement data can be obtained simply by pointing at the contour of interest. (If a contour has zero length and enclosed area, calibration of the study is required. A non-zero length with zero enclosed area indicates that a contour is an open contour. Only closed contours have enclosed areas.)

Trace provides for output of additional quantitative values using the Quantify menu commands or the Measure Tool. An representative application of the Measure Tool is measuring the lengths of processes which extend through several sections. When the process is oblique to the plane of section, the length calculation takes into account both the in-plane translation and the section thickness to obtain the correct Euclidean distance measurement.

To measure the length of a sometimes obliquely sectioned dendrite, follow this procedure:

- Go to the section in which the measurement is to begin.
- Place the measure tool cursor in the center of the dendrite cross section.
- Click the left mouse button but do not move the mouse -- leave the cursor at the center point.
- Page up (or down) to the next section in the series.
- If the cursor has moved out of the object, return it to the center. Otherwise, do not move it.
- Click the left mouse button and do not move the cursor.
- Continue paging and clicking until the entire length of the object is traversed.

At the end of this procedure, the length of the object will be displayed in the Length field. Note that the accuracy of this measurement depends on accurate calibration data, both for x- and y-directions within a section and for the average section thickness of the study.

Determine Section Thickness

Rather than trying to determine the exact thickness of each section, IGL Trace assumes a single average section thickness for the series. To determine or verify the section thickness of a study one can use the following procedure. This procedure requires that a cylindrical or spherical object be sectioned in the study.

First, calibrate the study if is not already calibrated such that measurements can be made within a section, i.e. in the plane of section. Then, locate the section with the central portion of the cylindrical or spherical object such that the diameter of the object can be measured in that section. Use the Measure Tool to measure the diameter of the object. Record this value.

Now count the number of sections in which the object appears. (For cylindrical objects such as mitochondria, this assumes that the long axis of the cylinder is parallel to the plane of sectioning.) The number of sections it appears in, times the section thickness, gives an approximation to the diameter which can be used to determine the section thickness.

$$\text{section thickness} = \text{diameter} / \text{number of sections}$$

By computing this value for many objects throughout the series and averaging the result, a reasonable estimate of the average section thickness is obtained.

Counting Objects

Trace can be used to count objects in physically or optically sectioned material. The basic idea is to mark objects with the contour drawing tools and then have Trace report the number of objects using the Quantify menu items. One approach is to use a sampling method to determine the number of objects present in sectioned material. To obtain unbiased results when counting by sampling several techniques are available. For a discussion of some of these techniques and the method of using them in IGL Trace, refer to J. C. Fiala and K. M. Harris (2001) Extending unbiased stereology of brain ultrastructure to three-dimensional volumes. *Journal of the American Medical Informatics Association* **8(1)**:1-16.

3D Reconstruction

An essential function for the Trace program is to provide data (in the form of named contour traces) which can be used to generate a three-dimensional reconstruction of serial sectioned objects. Once contours have been traced in each section, appropriately named, (each object must be consistently named throughout the sections,) and SAVED, then the user can obtain a reconstruction in one of two ways. The method of reconstruction chosen will depend on the desired fidelity of the 3D visualization.

One method is to export the contours to another software program where the detailed reconstruction and rendering can be performed. Such programs must be able to take in a serial set of planar contours and from this, either automatically or with user assistance, produce a rendering of the 3D structure. Some packages which have been used with IGL Trace in this manner are DesignCAD 3D, Interactive Computer Analysis and Reconstruction (ISG Technologies), and NUAGES (Bernhard Geiger). The specific export format used from IGL Trace will depend on the 3D software package used. For example, contours can be exported to NUAGES contour file format and then this contour file can be imported into the NUAGES program for 3D reconstruction.

An alternative method for 3D reconstruction is to select one of the operations from the 3D Menu. These operations generate a 3D reconstruction in VRML. When IGL Trace is properly configured with a program for viewing VRML, a 3D display can be invoked in a new window by IGL Trace. When using the 3D Menu operations a representation of the reconstruction in VRML format will be written to a .wrl file in the same directory as the study. This file can be viewed using Web browsers capable of interpreting VRML, such as Netscape with Silicon Graphics' CosmoPlayer plug-in.

New

To create a new study, select this menu item and enter the name for the new study in the dialog box. The new study will be created in the selected directory.

Open

Selecting this menu item opens a dialog box in which the user can select the name of the existing study which is to be opened. The user can browse the file system for studies. The names of the studies found in the current directory are displayed in the dialog box.

Import

These menu commands allow an image stack or movie file to be imported into IGL Trace. The images are broken out into separate files named according to IGL Trace conventions (_study.0, _study.1, ...) and saved in IGL image file format. The individual sections can then be processed as with any other IGL Trace study.

Intervision

Import a confocal series obtained from the Noran OZ confocal microscope in the InterVision data file format. InterVision data files contain a series of confocal images obtained using the Intervision z-series protocols. The x- and y-calibration information and the section thickness are also extracted from the InterVision file header and written out to a .clb file so that the series is correctly calibrated.

Generic File

Import a stack or movie file with a generic format consisting of a header of known size, followed by the 8-bit/pixel images of known width and height. The user is asked for the required parameters of header size, image width and image height.

Hide

Selecting this menu item causes the image data associated with a section to not be displayed. Instead, a uniformly gray background will be displayed in the image display area. Hiding the image display will speed up the browsing of contour data, since large image files will not need to be read from the disk. Also, if image data is not present for a given section but contour data is, hiding the image data will prevent the generation of messages regarding the absence of image data files. When selected, this menu item switches to Show.

Contrast

Selecting this menu item invokes the Contrast window. In the contrast window the user can modify brightness, contrast, and change the threshold level. In addition, several image operations can be performed as described below. In all cases, the original image data is not modified by these operations -- the contrast parameters are retained with an IGL annotated image and applied to the color table rather than to the image pixels themselves. Thus, the original data can be recovered from these operations by restoring the color table. However, if the original image is not in IGL Annotated format the contrast settings are not savable without saving the entire image.

Note: Contrast manipulation is valid only for grayscale images. To produce correct results on most color images, use the Image Filtering operation To Grayscale before manipulating contrast.

Threshold

Thresholding causes the image to be converted to a binary (black and white) image with all image points above the threshold being colored white, while those below threshold are colored black.

Reverse

Reverse the color table, making dark points light and light points dark, i.e. take the negative.

Linearize

Make the color table linear such that pixels with intensity zero appear black, pixels with intensity 255 appear white, and all intermediate values are mapped to a linear grayscale.

Equalize

Apply histogram equalization to the color table. This operation is useful for revealing contrast in what might originally appear as a uniformly dark or light image.

Auto Save

Automatically save contrast changes to file as needed. AutoSave will update the header of an IGL Annotated file, storing the new contrast settings without modifying the actual image data itself. But if the image format is not already IGL Annotated, then AutoSave will convert the image to IGL Annotated in order to save the contrast settings. This means that the image data will be rewritten completely and stored in its current state. Thus, if Image Filtering operations have been performed and the image file format is not IGL, AutoSave will replace the original data with these modifications.

Retain Settings

Retain and apply the present contrast settings to the next section visited.

Image Filtering

Image processing operations are selected from this submenu. Image processing operates on the image data of the current section, modifying the version of this data currently in the computer's memory and displaying the result on the screen. The original image data is only modified if the section image is subsequently saved. The image can be saved by selecting Save from the Section Menu. In many cases, image filtering will be desired only as a means to facilitate tracing. Once tracing is completed, the user will want to return to the original image data. This can be done by not saving the image and using the Goto command to reload the section image file from disk.

During processing, which may take some time depending on the image size among other things, an hourglass is displayed in place of the usual cursor. While the hourglass is displayed, user input is not accepted. The application will appear to be "dead" until processing is complete and the results are displayed on the screen.

Convolution

This processing implements a linear convolution of a user-specified convolution kernel with the current image and displays the result. The user is prompted to select the kernel mask (.mas) file. Several kernel files are provided with IGL Trace. For example, a file called "edges.mas" implements an 11x11 Laplacian of Gaussian kernel which would serve to eliminate illumination gradients and highlight contrast changes in the image. For more details on customizing kernel files see File Formats.

The convolution process rescales the output to 0-255, the range of grayscale values available in the original image. In most cases, convolution will need to be followed by manipulation of image contrast to emphasize the range of grayscales containing the most information. This can be done using the Contrast dialog.

Correlation

Correlation processing implements a correlation between the image and a subregion of the image delineated by a user specified contour. The user inputs the contour name of the contour which bounds the region of interest. The operation then uses correlation to locate matches to this region over the entire image. The result of the operation is a new section image indicating the degree of match at each point in the image. Matches are indicated by regions of high image intensity (i.e. white) while a failed match condition is denoted by low intensity (i.e. black).

By thresholding the output of the correlation, the user can select the match regions. Tracing a contour in each match region allows the exact point of match to be calculated by computing the centroid of this contour. The contour used to delineate the original subregion can be mapped onto the centroids of these match point contours by using Replace with... from the Contours Menu.

Median

Compute and display the median image for the current section. This operation is useful for removing scattered dots from the image and creating more uniform image regions. Uniform regions are easier to trace using the automatic tracing tools than clouds of scattered dots.

To Grayscale

Convert a color image to grayscale. Much of the functionality of IGL Trace is based on the assumption that the image is an 8-bit grayscale intensity image. Although Trace accepts and displays color images, these will need to be converted to grayscale for almost all operations except manual tracing.

Export Image As

This command allows the image data for the current section to be saved into another file with a different file format. Current exportable image file formats supported are Windows Bitmap, PCX, and TIFF.

Goto

This menu item activates a dialog box which allows the user to select a section number from those available in the current study. After a section number is selected, the image and contour data files for that section are loaded from disk, and the selection is made the current section.

Unlike the previous, predecessor, and successor commands, Goto always loads the image and contour information from disk each time it is invoked. So Goto can be used to restore the original image data display, for example after an image filtering operation. However, this means that occasionally the previous and current contours, both of which are held in memory are from the same section. When a new section is visited, a message of the form “Two versions of section contours are active. Keep current ones?” will be displayed. Responding “Yes” will result in the current contours becoming the previous contours and the older version being deleted from memory. A “No” response will keep the previous version loaded instead of the current one.

Calibration

Selecting this menu item opens and displays the calibration section (section 0). The user is then given the option of viewing or modifying the calibration data associated with this section. This data is the basis for spatial measurements of all the section data in a study. The user may exit the calibration process without modifying the calibration file by selecting CANCEL in one of the dialog boxes. When the calibration data is modified, the previous calibration data file is saved in a backup file with a tilde as the first character.

Contour Lengths

Before electing to enter or modify calibration data, the user should first trace contours in the calibration image which can be used to determine spatial extents in the x- and y- directions. This can be done by following the calibration procedure. Note that calibration contours should be given unique names because the user will be asked to enter the spatial extent of each contour by name.

Section Thickness

In addition to calibration contour lengths, the user is asked to enter the section thickness for the study. The section thickness is the distance in microns between serial sections. Trace assumes a *a single average section thickness* throughout the study. Section thickness can be estimated using the procedure described here.

Although only the calibration contour lengths and section thickness data are necessary for calibration, the user is also given the opportunity to enter additional descriptive information that will be recorded in the study calibration file. The calibration file is an ASCII file readable with any text editor. See File Formats for more details. The optional calibration information includes:

Dpi in X

Since most digitization is now done by desktop scanners, options are provided for recording the associated acquisition parameters, one of these being the resolution used during the scanning process along one axis. This is the dots per inch (dpi) in the x-direction.

Dpi in Y

The dots per inch (dpi) in the y-direction used during digitization. Note that the Dpi parameters are also recorded as 12-character strings such that any other acquisition parameters could be entered here instead. The dpi values are not used in the calibration in any way; they are simply recorded for future reference.

Comment

A string of up to 48 characters to describe the date, equipment, and other conditions of the digitization process.

Hide

Selecting this menu item causes the contour data associated with a section to not be displayed. If contour data is not present for a given section but image data is, hiding the contours will prevent the generation of messages regarding the absence of contour data files. When selected, this menu item switches to Show.

Name Table

Selecting this menu item invokes the Name Accelerator Table window in which the user can see the accelerators for various contour names.

Each study has its own Name Accelerator Table which is initially blank. The user can define accelerator keystrokes for a contour name and color such that the keystroke will be all that is required to change contour name and color while tracing. To define an accelerator, check the Modify Mode box in the Name Table window. Then move to the Trace window and set the desired color and contour name. Hold down the control key and the desired letter to store the accelerator values. When all Name Table entries have been made, return to the Name Table window and uncheck the Modify Mode box to enable the name accelerators.

To use a name accelerator in the Trace window, hold the control key and hit the desired letter. The default color and contour name will be changed to the value stored in the Name Table.

Trace Regions

This command attempts to automatically contour each distinguishable white region found in the image, giving them the current default contour name and color. Regions are distinguished according to the current threshold value, which can be modified through the Contrast dialog.

WARNING: Trace Regions will attempt to locate and trace out EVERY above threshold region in the ENTIRE image. For large images this can take a very long time. Also, the number of contours created can be very large. Proceed carefully when using this command. Use the threshold operation from the Contrast dialog to verify that there are a reasonable number of white regions to be traced prior to selecting Trace Regions. To trace one region at a time, use the Wildfire Tool.

Wildfires

These submenu items allow the user to tune the performance of the Wildfire tracing algorithm.

Stop at Contours

When checked, Wildfires will stop before contacting existing contours. When unchecked, Wildfire contours will ignore and cross contour boundaries.

Spread

This submenu contains settings for regulating the spread of Wildfire contours:

More
Wildfires spread as readily as possible, moving to any of the eight-connected neighbors of the specified point or starting contour.

Default
Wildfires spread to any of the four-connected neighbors of the specified point or starting contour.

Less
Wildfires are least likely to spread in this mode, since there must be three adjoining white pixels for spreading.

Copy

Selecting this menu item copies the contours from another section to the current section. The copied section contours are added to the contour set for the current section. The data on disk is not modified until the user performs a Save of the contours for the current section.

To copy contours from a different study, try exporting contours as a raw ASCII file and then importing into the desired study.

Replace with...

This command will place a user-selected contour at the centroid of each named contour found in the current section. Both the selected contour and the replaced contours must exist in the current section prior to invoking the command. Changes do not become permanent until the user saves the contours. The replaced contours can be immediately recovered by using the Insert key to undelete them.

Rename...

This command renames all the contours with a given name in thecurrent section and throughout the series. The command first executes on current section contours only. Running the command a second time will cause it to operate on the remaining secitons of the study.

Delete All...

This command deletes all the contours with a given name in thecurrent section and throughout the series. The command first executes on current section contours only. Running the command a second time will cause it to operate on the remaining sections of the study. The deleted contours in the current section can be immediately recovered by using Insert.

Clockwise...

This command reorients all contours with a given name in a particular range of sections. All closed contours are reoriented, if necessary, to be clockwise.

Recall that clockwise closed contours are interpreted as the exterior boundary of the object, while contours drawn in a counterclockwise orientation are interior boundaries. Counterclockwise is used onlyto outline holes in the interior of clockwise contours.

Find

Selecting this menu item allows the user to find the first occurrence of a contour name. The user is queried for the contour name to search for. If an incomplete contour name is given, Find will locate the first contour that begins with the given string. For example, the search string “PSD” will match contours with names “PSD”, “PSD13”, “PSDGAP”, etc., but not “PS” or “psd”. Find first looks in the current section. The first contour found which matches the search string is made the selected contour just as if it was selected using the Select Tool. The user can then use the toolbar to Delete or otherwise modify the found contour.

If the search string does not match any contours in the current section, or if the study does not currently have a section open, the user is prompted for a range of section numbers in which to search for the contour name. In this case, Find reports the section number of the section that contained the first occurrence to match the search string.

Save Contours

Selecting this menu item saves the current contour data to disk. When contour data is saved, a backup file is made of the old contour data. To recover this data you'll need to manually delete the contours file and rename the corresponding backup file.

Export As...

The contour information is written into a foreign contour file format using this command. In most cases, contours for the entire study are exported at once. However, in the case of 2D export formats, only the contours from the current section are exported. The available formats are briefly described below. Detailed information on the file formats is available in the File Formats section.

IGL File

IGL File format was used within the former Image Graphics Laboratory at Children's Hospital to transport contour data to the ICAR system for 3D reconstruction. Selecting this option additionally requires generation of a .map file which contains the mapping from contour names to specific level values in ICAR. Once a .map is created and saved, it can be used to generate the IGL File which is given the .igl suffix.

Boulder HVEM File

The Boulder HVEM File format has been associated with the UCSD reconstruction systems developed by Steve Young and Mark Elisman. This option generates a REC.CNF parameter file and a .REC contour file.

Universal Contour File

The Universal Contour File format was developed at the Laboratory of NeuroImaging (LONI) at UCLA for use with reconstruction systems developed there. This option generates a .ucf contour file.

ROSS Contour file

This option generates a .asc contour file in the format used by the Reconstruction Of Serial Sections (ROSS) program at NASA Ames Research Facility.

AutoCAD DXF File

This option generates a .dxf file which will allow the contours to be imported into AutoCAD and most other CAD and 3D graphics programs.

Nuages Contour File

Selecting this option will result in the generation of one or more .cnt contour files which can be importing into the NUAGES program of Bernhard Geiger. A .cnt file will be generated for each object in the study. These files can be individually imported in NUAGES and the resulting files, in Open Inventor format, can be concatenated and edited to produce the desired 3D visualization of multiple objects.

2D Raw ASCII File

The contours for the current section (if visible) are output in raw image coordinates in ASCII format. A .raw file will be generated with the study name. This file can be imported into another section of any study by using the Import command from the Contours menu.

2D HTML Image Map

The contours for the current section (if visible) are output as an HTML image map in ASCII format. Each contour generates a *poly* shape with the *coords* being the contour points in image coordinates and an href of the contour name with a .htm suffix. A .htm file will be generated with the study name.

Quantify 2D

This item contains a submenu of commands for obtaining 2D information about the traced contour data. Most commands extract information about selected contours in a section. For example, selecting a contour with the Select Tool and then requesting a Count will result in a count of all contours with the same name as the selected one. If a command is issued without first selecting a contour, a contour name will be requested along with a section number range. The program will then search those sections and quantify all occurrences of that contour name.

Count

This menu item returns a count of the number of occurrences of the contours in the current section which have the same name as the name of the selected contour. The selected contour is included in the total. If no contour is selected, any contour name can be counted in a specified range of sections.

Area

Selecting this menu item returns the total 2D area enclosed by the contours in the current section which have the same name as the name of the selected contour. The selected contour’s area is included in the total. If no contour is selected, the total area for any contour name can be computed from a specified range of sections. NOTE: Areas of counterclockwise contours are subtracted from the total area, in effect removing the areas of "holes".

Centroid

This menu item reports the centroid of the selected contour or the centroids of each contour found in a range of sections specified by the user.

Report

The Report command outputs 2D quantities in a text file format capable of being imported into a spread sheet. However, the text file is automatically opened in Notepad so the file can be immediately reviewed on the screen.

Points, Lines, Planes and Slabs

Selecting one of these menu item will invoke a 3D display of the contours in a separate window using the VRML viewer specified in trace.ini. A representation of the reconstruction in VRML format will also be generated to a .wrl file. This file can also be viewed using Web browsers and other tools capable of interpreting VRML.

Some of these commands request an "Error Bound for Reconstruction" which is used to determine how to reduce the number of points in the contour before generating the VRML output. The nature of the current contour simplification procedure is such that the contour reduction can produce some overlap or loops in certain contours. In these cases, the contour may not appear in the output display, especially when output is Planes. This problem will be addressed in a future release. It can be avoided for the present by selecting the "Max. error in pixels" to be zero. Note that a similar statement can be made for the "Simplification Amount?" parameter used in the Export As command.

Points

This menu item generates a 3D representation of the centroids of particular named contours. The 3D representation can be a VRML sphere or cube, or simply a flat triangular face with the same centroid. This function provides a simple method for generating 3D point objects corresponding to contours.

Lines

This menu item generates a colored 3D representation of the contour lines in space for the selected object. If no contour is selected,all objects in the specified section range are displayed.

Planes

Selecting this menu item produces a 3D representation in which open contours are lines and closed contours are colored planar regions. Serial sections of each object are shaded in depth to facilitate 3D visualization.

Slabs

This menu item generates a 3D VRML representation in which each contour is given a single section thickness. In this representation, a contour forms a colored slab in space.

Surfaces

This operation generates a 3D VRML representation of the surface of an object as a set of planar triangular surface patches. In its current preliminary form the operation produces a surface for one object as specified by the set of contours with a given name.

The algorithm assumes that all contours are closed and free of loops. The exterior of the object is specified by closed contours with a clockwise orientation, while interior surfaces, i.e. holes, are given by closed contours with a counterclockwise orientation. Any counterclockwise contours in a section should be completely enclosed by a clockwise contour. Violations of these conditions may result in an undesireable effect.

If the operation produces gaps, missing contours, or other errors in the reconstruction, try setting the Max. Error parameter to a larger value. Larger Max. Error values produce reconstructions with fewer surface patches. Alternatively, check for problems with the contours. Make sure that all exterior contours are closed and clockwise. Try to eliminate any loops or overlapping parts of contours. Contours which create problems for the reconstruction may ultimately need to be redrawn or simplified.

Selecting the Surfaces menu item brings up a dialog box in which the user can select several options for the reconstruction. In desired, all contours can be forced clockwise during the reconstruction by the selecting the “Make Clockwise” item. To automatically eliminate loops, the “Expand” or “Shrink” options can be selected. These items apply the shrink and expand operations as found on the tool bar to be applied to each contour during the reconstruction. Note that when Expand is used, the contours are also forced clockwise. Shrink will allow clockwise and counterclockwise contours, but may not be successful in all cases, particularly when the contour is very narrow at one or more points.

Quantify 3D

The 3D quantify commands extract information about 3D objects defined by contours in the study. The component traces of an individual 3D object are grouped by a common contour name. For example, if a contour in a given section is named DENDRITE, then other contours named DENDRITE in that section and adjacent sections are considered to be part of the same 3D object.

Note that the 3D quantities computed here are obtained by multiplying the appropriate 2D quantity by the average section thickness for the study. In other words, the surface area of a single contour is the length of the contour times the section thickness. The full 3D quantity is the sum of many such parts. This approach yields an approximate answer.

The origin for all 3D coordinates reported is the upper-left corner of the first (non-zero) section in the study, with positive z in the direction of higher section numbers. The origin remains fixed no matter what section number range is selected for analysis. Thus, coordinates from two different 3D computations can be directly compared as long as there has been no change in the first non-zero section number. The object extent in the z-direction may be negative since a section thickness is considered to the be half above and half below the (2D) image plane.

Surface Area

This command reports the surface area (in square microns) for the 3D object of which the selected contour is a part. The surface area includes the product of length and section thickness for all contours in the object, plus the sum of the enclosed areas of the contours in thefirst and last sections.

Volume

This command reports the volume (in cubic microns) for the 3D object of which the selected contour is a part. Volume is computed by the sum of enclosed area of each contour in a section times the section thickness. Counterclockwise contours produce negative areas which decrease the volume. Thus, "holes" are subtracted from the total volume.

Object Extent

This command reports the minimum and maximum x, y, and z values (in microns) for the 3D object of which the selected contour is a part.

Object Midpoint

This command reports the center point (x,y,z) of the Object Extent for the 3D object of which the selected contour is a part.

Report

This command generates a text file report of 3D quantities.

Select Tool

The Select Tool is used to select contours from those displayed on the screen. When this tool is active the cursor is the standard Windows arrow cursor.

To select a contour with this tool, move the mouse into the section display. The contour nearest the cursor position will be reported in the Status Bar. When the desired contour is reported, click the left mouse button. This selects the contour, causing it to be highlighted. The Contour display field should now continuously report the selected contour as the mouse moves over the display. To deselect the contour, reselect the tool. This can be done by either clicking the tool button or by clicking the right mouse button.

Pencil Tool

The pencil tool can be used to trace contours on the section image. To trace contours, first select the pencil by clicking on the button with the left mouse button. Then move the pencil cursor to the image display. Click the left mouse button to begin the contour. Contour points can be added by moving and clicking the left mouse button or by holding the left mouse button down and moving the mouse around the display. A pencil contour can be terminated by selecting another tool or by holding down the control key and clicking the right mouse button. For example, a pencil contour can be simultaneously terminated and converted to a closed contour by selecting the Close button.

The Backspace key may be used to erase contour points, starting with the last point drawn. This function is available while tracing with the pencil tool and may also be applied to a contour which was previously drawn and now has become the currently selected contour.
Picking up the pencil while there is a selected contour allows you to continue tracing this contour. The selected contour is opened and tracing continues from the last point drawn.

Ellipse Tool

The ellipse tool is used to facilitate the creation of elliptical closed contours. Trace elliptical contours by selecting the ellipse icon and then holding down the left mouse button and moving the mouse on the image display. When the left mouse button is released the elliptical contour will be created. A new contour can then be draw without reselecting the tool.

Measure Tool

The measure tool is used to make spatial measurements within and between sections. Activate the tool by clicking on the segmented ruler icon in the tool bar. To make a measurement, click on a spot in the image with the left mouse button, move to a new spot and click again. The length of the drawn line segment is shown in the Length field of the screen display. To add an additional line segment to the total computed length, click in the image again. Clicking the right mouse button enables the user to start a new one.

Multisection lengths can be measured as well. This is useful for measuring the length of objects which extend through several sections. See the description of the Measurement procedure.

Split Tool

The Split tool allows a contour to be cut at point selected by the user. If the contour is a *closed* contour, Split allows the beginning and end of the contour to be relocated to the cut point, rather than remaining where drawing initially began and ended.

To use this tool, select it and move the cursor inside of the contour that will be cut. Click and hold the left mouse button. The contour selected for cutting will now be highlighted. Now move the mouse to the point on the contour where a break is to be made and release the left mouse button. The contour will be broken at an original drawn point closest to the cursor.

If the contour was originally closed, it is converted to an *open* contour, with the end and start at the cursor point. If the contour was already open, it is broken into two separate contours at the cursor point, one of which will become the selected contour. Once the contour is opened by splitting, the Backspace key may be used to delete contour points starting at the break point, or the Pencil Tool may be used to add to the contour.

Close

A contour in IGL Trace may be either open, in which the end is not attached to the beginning, or closed, in which the end is attached to the beginning. Open contours have no enclosed area. Selecting the Close tool converts the currently selected contour, if any, to a *closed* contour. To reopen the contour, use the Split Tool.

Zoom Tool

Use this tool to zoom a rectangle region of the image. After selecting the tool from the tool bar, move to the upper left corner of the region to be magnified. Press and hold the left mouse button down, while moving the cursor to the lower right corner of the region to be magnified. Releasing the left mouse button causes the selected region to be zoomed. To unzoom the image, use the End key. To rezoom, use the Home key.

When the image is zoomed such that the whole image will not appear in the display area, scroll-bars appear at the edge of the display area. These allowing panning the zoom region around the whole image. Clicking in the scrollbar (but not on the arrows or thumb position marker), page the zoom region a distance equal to the size of the zoom region, if possible.

Wildfire Tool

The Wildfire tool provides a means for automatically tracing the dark boundary of a light region. First, display the section image in which tracing is to be done. Then select the Wildfire tool and place the match cursor in the interior of the region to be traced. Click the left mouse. The closed contour bounding the region will be created and displayed.

To control the grayscale value that marks the boundary of the region, use the Threshold slider in the Contrast window. The Threshold between light and dark is where the Wildfire contour will be drawn. Turning on Threshold (check box) in the Contrast window will clearly indicate where the boundary will be drawn.

To control the spread of the Wildfires from the desired region, use the settings in the Wildfires submenu of the Contours Menu. When the Wildfire contour includes unwanted adjacent regions, try selecting the Wildfires--Spread--Less item to eliminate this problem. Another way to prevent unwanted spread of wildfires is to select Stop Wildfires at Contours (the item will be checked), and then drawing temporary contours in the places where wildfires should be blocked.

Often regions, or their boundaries are not solid regions of uniform grayscale, but are clouds of dots. Consolidate these clouds into uniform grayscale regions prior to tracing with Wildfire. This can be done by using Image Filtering. Median filtering is particularly effective at consolidating clouds of dots in solid regions of uniform grayscale.

Wildfires can also be produced from existing contours. This is useful for guiding wildfires through a problematic region while letting the easy part be handled automatically. To use this feature, draw a contour with any drawing tool. Then, with the contour selected, click the wildfire tool button. Parts of the contour which are on black regions will not move. But the parts of the contour on white regions will ignite, spreading to the borders of the region.

Join Tool

The Join Tool allows two contours to be connected together. To use it, select the tool and move to a point on the first contour. Press and hold the left mouse button. Verify that the desired first contour is highlighted. Move to a point on the second contour and release the left mouse button. The two contours will be combined into one contour as follows.

If both contours are *open*, they will be connect head to tail, the tail of the first contour attaching to the head of the second contour such that the new contour has the head of the first contour and the tail of the second contour. If one or both contours are *closed*, the closed contour(s) will be opened at the point touched and then connected head to tail as with two open contours. The final contour will have the characteristics of the first contour, including its closure. That is, if the first contour was closed then the final contour will be closed, Thus, if both contours are closed, have the same orientation, e.g. clockwise, and do not overlap, then the final contour will be a closed contour without loops. If the two original contours differ in orientation, or one contour is inside of the other, then joining them may produce loops. Use the Orientation command from the Contours menu to adjust contour orientation prior to joins.

Point Tool

The Point tool is used to create small, circular contours centered at a point in the image. Select the tool by clicking the button and then move to the image point where the point cursor is to be created. Click and release the left mouse button to create the point contour.

Line Tool

The Line tool makes straight line contours between two points. Select the first point by clicking and holding the left mouse button, then drag the mouse to the second point and release the left mouse button. A straight contour will be made between to the two points.

Colors

The colored tiles on the toolbar are used to select colors for contours. The current (default) color is marked by an X. To select a different color, depress the left mouse button while the cursor is on the desired color. When a color tool is selected, the default contour color is changed to that color. Any drawing tools subsequently selected will employ the default color. An existing contour's color can be modified by first selecting the contour with the Select Tool and then clicking on the desired color.

Alternative colors can be selected from the Color dialogof the Contours menu. A color so selected will be applied to the selected contour and will becomethe default color for any new contours created.

IGL Trace File Formats

Complete file format specifications are given for all IGL Trace related files. Conventions used when describing numeric ASCII fields: f=floating point, d=integer, s=string. All other values are produced exactly as given. For binary files: byte=char=8-bit character, short=16-bit integer, long=32-bit integer, float=32-bit IEEE floating point.

3D Output File Format

Three-dimensional geometries are output as Virtual Reality Modeling Language (VRML) files. These files are ASCII files compliant with the VRML 1.0 Specification. They are given a .wrl extension and can be read by many WWW browsers and other 3D display programs. A typical file might look like:

```
#VRML V1.0 ascii
# 3D scene generated by IGL Trace from study s

Separator {
  Separator {  # object: s, sections d - d
    Material { diffuseColor f f f
               ambientColor f f f
               emissiveColor f f f }
    Coordinate3 { point [
                   f f f
                  .
                  .
                   ] }

    TransformSeparator {
      ShapeHints {
                  vertexOrdering CLOCKWISE
                  shapeType UNKNOWN_SHAPE_TYPE
                  faceType CONVEX
                  creaseAngle 0.0 }

       IndexedFaceSet {
               coordIndex [
                     d d d -1
                     d d d -1
                     .
                     .
                      ] }
    }
  }
}
```

Calibration File Format

V8 calibration files are ASCII data files with .clb suffix. Any line in the file that does not start with one of the following ASCII strings is ignored:

xy:

Lines that begin with xy: are calibration lines and consist of 5 fields as in: ‘xy:3.92   54 73 313 73’. The first field following the xy: keyword is the length of the calibration line in units of microns. The next four fields determine the endpoints of the calibration bar. The second and third fields represent the coordinates of the beginning of the calibration bar. Similarly, the fourth and fifth fields represent the coordinates of the end of the line segment. Thus, the above example represents a calibration bar that is 3.92 microns long and runs from pixel coordinates (54,73) to (313,73).

section thickness:

Lines that begin with this string specify the section thickness of the study as a floating point ASCII string following this descriptor: “section thickness: 0.06700”.

dpi in x:

This line gives the dots per inch used on the scanner at the time the study images were digitized. Although this information is not used by the software, it is recorded here to provide the user with a way to conveniently store and keep this information with the data files. It may be important if the user wishes to acquire images later with the same spatial dimensions.

dpi in y:

This descriptor is followed by the dots per inch of the digitization, for descriptive purposes only.

comment:

This descriptor is followed by an optional user supplied string for descriptive purposes only.

NOTE: Older versions of the calibration files (used with WinV8) may contain additional descriptive fields, such as “camera height:”, “camera type:”, and “station:”. These are ignored by the current version of IGL Trace.

Contour File Format

The V8 contour file is a binary file that contains the contour information. There is one such file per section. The name of the contour file consists simply of the base name of the study with a numeric extension that represents the section number. The file consists of a file header followed by a number of variable length records with one record per contour. Each record consists of header information that supplies the length of the record and the type of object it represents. Following the record header is the actual data for the object.

The file header consists of the 5 bytes: 0xFF 0xFF 0x56 0x38 0x1A plus a one byte version number. Files that do not start with this information are considered obsolete.

The record header is 13 bytes long organized as follows:

- short Size of the entire record
- 8 bytes Name of the object
- 1 byte Color of the object
- short Type of object

Here short indicates a positive integer represented by 2 unsigned char values in low byte-high byte order. Thus, the format does not currently allow negative numbers.

The rest of the record consists of data that are organized according to the type of the object. For OPEN and CLOSED contours, the data following the header consists of alternating x and y position of the contour line segments. That is, ‘short x0 short y0 short x1 short y1 ...’. Since the contour points are these special 2 unsigned char values, negative contour points are not representable.

Contour Import File Format

Single contours can be imported into a section from an ASCII file specifying the (x,y) positions of the contour points in image coordinates. In this format, which is the same as the 2D raw ASCII export format, the contours are represented as a series of (x,y) coordinates, given in image coordinates. The values are integers with (0,0) being the upper-left corner of the image as it is displayed on the screen. Unlike other contour file formats, no scaling or modification of the contour data is made during raw export. Each contour is preceded by a name of up to 8 alphanumeric characters on a new line. Then the contour points are given as the series of (x,y) points, each point on a separate line. When there is only one contour, the contour name is optional, otherwise the name strings are necessary to delineate individual contours.

```
s
d d
d d
d d
d d
...
s
d d
d d
d d
...
```

Dynamic Link Library Definition

You may create dynamic link libraries that give IGL Trace the ability to read arbitrary image file formats. Creating such a DLL and including a reference to it in trace.ini will give IGL Trace the ability to read image files with a format different from those built-in to IGL Trace. IGL Trace's access of the images will be no different that the other formats already built-in to IGL Trace. In addition, the description of the format will appear in the list of acceptable formats in the "Input Formats" item of the Help menu. To realize this, include a STRINGTABLE resource in your DLL with an ID of 1 associated with a descriptive string of not more than 30 characters. For example:

```
STRINGTABLE
{
 1, "LONI's "PIC" Type UIF Format"
}
```

To complete this concrete example, here's the entire DLL source written for the Borland C++ compiler for the "PIC" input format.

```
//////////////////////////////////////////////////////////////////////////////
#include <stdio.h>
#include <owl\owlpch.h>
static TModule *gModule;
BOOL WINAPI DllEntryPoint(HINSTANCE hInst, DWORD why, LPVOID) {
	if (why == DLL_PROCESS_ATTACH)
		gModule = new TModule(0, hInst);
	else if (why == DLL_PROCESS_DETACH) {
		delete gModule;
		gModule = 0;
	}
	return TRUE;
}
//	Here's the exported routine that produces a TDib from the file!!
TDib * _stdcall _export loadimage(TWindow *parentwindow, const string &filename)
{
	FILE *fp;
	TDib *outdib;
	int i, num, j;
	int depth, width, height;
	unsigned char buf[512];
	unsigned char *p;
	outdib = 0;
	if ((fp = fopen(filename.c_str(), "rb")) == NULL) return outdib;
	if (512 == fread (buf, 1, 512, fp))			// first 512 bytes contain header info
		{
```

```
    sscanf(buf,"<depth=>%d\n<width=>%d\n<height=>%d", &depth, &width, &height );
    if ( (depth == 8) && !(strncmp(buf,"<depth=>",8)) ) {
                                                            // create an 8-bit image
        outdib = new TDib(width, height, 256, DIB_RGB_COLORS);
                                                            // initialize color table
        for (i=0; i<256; i++ )
            outdib->SetColor( i, TColor( i, i, i ) );
        unsigned char *pImage = (unsigned char *)outdib->GetBits();
        while ( (num = fread(buf,1,512,fp)) > 0 )
            {
            for (j=0; j<num; j++) {                                     // set scan line
into dib
                *pImage = buf[j];
                pImage++;
                }
            }
        }
    }
  fclose(fp);                                                           // close file
and return dib
  return outdib;
}
//////////////////////////////////////////////////////////////////////////////
```

Note that the exported routine loadimage(...) is given the file name and must return either a pointer to a valid TDib containing the image read from the file or a NULL pointer indicating failure. The routine should not attempt to report error messages to the user but simply indicate failure by returning NULL, since Trace will interpret failure as the need to try other image formats.

To get IGL Trace to recognize and use the DLL, the DLL and trace.ini must both reside in the directory of the executable of the main program. Modify the InputFilters area of trace.ini, which might initially look like...

```
[InputFilters]
NumFilters=1
Filter1=myinput.dll
```

Increment the NumFilters parameter to indicate the addition of a new filter, and add a new filter line, such as...

```
[InputFilters]
NumFilters=2
Filter1=myinput.dll
Filter2=picinput.dll
```

Edge Mask File Format

The edge mask (.mas) files are simple ASCII files that contain the numeric values for kernels used in the convolution image filtering process. Convolution kernels are square two-dimensional arrays of real numbers which usually have an odd dimension. For example, the smallest possible symmetric square kernel would be a 3x3 kernel, such as the following simple “moran.mas”.

```
1 1 1
1 1 1
1 1 1
```

The kernel values are given in ASCII format, arranged in a two-dimensional array. This kernel in convolution leads to a pixel being replaced by the sum of itself and its surrounding eight neighbors.

Kernels can be any size up to 32x32. The speed of the convolution process is not effected by kernel size, so there is no particular advantage to using smaller kernels.

A great variety of operations can be devised by constructing the appropriate kernels for the convolution. For example, to extract horizontal edges in an images, the following 3x3 kernel could be used.

```
-9 -9 -9
18 18 18
-9 -9 -9
```

Similarly, to extract vertical edges use

```
-9 18 -9
-9 18 -9
-9 18 -9
```

IGL Image File Format

IGL Trace will accept image data in several formats, including many Windows Bitmap, GIF, TIFF, and JPEG files. (For a complete list of acceptable formats select the Input Formats menu item from the IGL Trace Help Menu.) The IGL image format known as "IGL Annotated" offers certain advantages, such as header contained annotation strings viewable from IGL Trace, and the storage and manipulation of color table parameters without altering the original image data. Under certain conditions, such as when saving or giving an image annotation, IGL Trace will convert a foreign format to the IGL Trace format. To view the converted images with another program you may need to export them to Windows Bitmap format.

The IGL image file format generated by the current version of IGL Trace/Align consists of a 512 byte header followed by the image data stored as a simple, flat 8-bit gray-scale image. The header begins with the 5 byte ‘‘magic number" string given by ‘\x1A\x0A\x05\x04’. Older image files, converted for use with V8 from the PANDORA system are often missing the "magic number" string in the first record. This is taken as an indication that the image data is stored as 512x512 interlaced image in which the 256 even image lines are stored in the first 256 records and the odd image lines are stored in the last 256 records.

IGL Annotated image file format header contains the following ASCII information:

byte 0: \x1A\x0A\x05\x04IGL Annotated. d d d d d d
byte 128: s

The integer fields in the first line are the width, height, flags, brightness, contrast, and threshold parameters respectively. The flags parameter contains the following bit components:

bit 0: 1 => EQUALIZED
bit 1: 1 => THRESHOLDED
bit 2: 1 => REVERSED
bit 3: 1 => LINEARIZED

At the 128th byte of the 512-byte header, the 128-byte long annotation string begins. The remaining 256 bytes of the header are reserved for a possible future use as a color table.

The binary data immediately follows the 512-byte header:

byte 512: 0x00 0x00 0x00 0x00 ... 0x00
byte 512+width: 0xff 0xfe 0x0f 0x00 ... 0x01
byte 512+2*width: 0xe0 0x40 0x00 0x32 ... 0x0a
.
.
byte 512+(height-1)*width: 0x00 0x00 0x02 0x0b ... 0x00

Intervision File Format

Intervision data files containing time- or z-series confocal images are a special instance of SGI Movie files. These binary files contain an 8-byte header ‘MOVI\x0\x0\x0\x3’ followed by a sequence of rectangular images. The first image area immediately follows the 8-byte header and contains the parameter information for the confocal acquisition and for the rest of the file format. The fields located in this first image area are denoted by an ASCII string followed by the parameter value. Some important parameter fields are

WIDTH	d
HEIGHT	d
__DIR_COUNT	d
NI_Xdimension	f
NI_Ydimension	f
NI_Zdimension	f

The exact location of these fields is variable and the space between them is variable, but they always occur in the above order.

Every image in the file is WIDTH*HEIGHT bytes long, one byte per pixel. The first data image is located WIDTH*HEIGHT+8 bytes from the start of the file.There are total __DIR_COUNT data images in the file, in sequential order.

Contour Export Formats

The foreign contour file formats, as described in the page on the Contours Menu Export As command, are described here as they are written by IGL Trace.

IGL File
Boulder HVEM File
Universal Contour File
ROSS Contour File
AutoCAD DXF File
Nuages Contour File
2D Raw ASCII File
2D HTML Image Map

IGL File

The IGL export format is used to export reconstructions to an ICAR system in the Image Graphics Lab. Generation of the binary IGL file utilizes (for historical reasons) a second ASCII file known as the .map file. This file contains a list of the contour names in the study along with two parameters designating the contour's "level". These parameters should be identical and between 40 and 160 inclusive. Contours that are inside other contours should be given larger values than the contours which enclose them.

The IGL file itself is a binary, indexed file which contains the exported contours for the ICAR system. This .igl file consists of four sections:

1. A file name header that contains global scaling information.

2. A Contour name table.

3. A section index that contains information about the location of various sections within the file.

4. Section data.

File Header

The first 16 bytes of the IGL file comprise the file header. This header consists of:

short Igl version number
float X global scale factor
float Y global scale factor
float Section thickness
short Number of Names in the name table

The X and Y scale factors have units of pixels per micron.

Contour Name Table

The Contour Name Table immediately following the 16 byte file header consists of 13 bytes of information for each name. The number of names is given in the file header. The 13 bytes are organized as follows:

8 bytes: Contour Name
short: Identification number
short: order parameter
byte: gray-scale

The identification number has no meaning outside of the IGL file itself. The order and the gray-scale parameters have application defined meanings.

Section Index

The section index immediately follows the contour name table. It consists of:

short: number of sections in the index

short: first section number
long: location of the first section
short: second section number
long: location of the second section
.
.

The section number and its location are repeated for every section. The location of the section is the byte offset from the beginning of the file.

Section Data

The data for each section follows the section index. Each section consists of some header information followed by the data for each object of the section. The section header consists simply of the 18 bytes

short: The actual section number
16 bytes: Reserved for the future

The size of the section is not specified by the header. Instead, the size may be deduced from the offsets specified by the section index. The actual data follows the section header. The data consists of a header for each object of the form:

long: size of the data plus header
short: identification number
byte: type

The size of the data includes the size of the data header. The identification number maps the data to a name as specified in the contour name table and the type parameter specifies the type of the object. The format of the actual data depends upon the type of object it represents. For open or closed contours, the data is laid out in a series of shorts that correspond to the (x,y) coordinates of the contour.

Boulder HVEM File

When contours are exported in the Boulder HVEM format two files are written. The first is an ASCII file named REC.CNF that contains the following line:

0 512 512 0 0 0 0 0

The second file is a binary file with a .REC suffix which contains the contour data. First, there is a 80 character comment field followed by the following fields:

short	/*index to last directory entry. lastdir +1 = total entries */
short	/*last plane drawn */
short	/*last object drawn */
unsigned char	/*last type drawn */
long	/*next free byte on file */
unsigned char	/*directory full flag */
unsigned char	
unsigned char	/*file aligned flag=1 (aligned) */
short	/*maximum number of directory entries */
short	
short	
short	
float	
float	/* tissue mag in K */
float	/* section thickness in microns */
short	/* x center of gravity in tablet units */
short	/* y center of gravity in tablet units */
short	/* (maximum plane number + minimum plane number)/2 */
short	/*number of deleted directory entries */

This is followed by 2000 directory records. Each directory record points to the location in the file of an individual contour. Real values are only found in those records needed to represent the contours of the series. Each record has the following format.

short	/* plane number */
short	/* an arbitrary object # */
unsigned char	/* type is a number 1-255 indicating structure membership */
unsigned char	/*=1 if data represents points */
	/* =0 if data represents a contour */
unsigned char	/* number of 100 point blocks required for contour = 0 for NULL plane */
long	/* byte offset from begining of file to contour dataheader */

Each contour consists of a header:

unsigned char	/*align=1 */
unsigned char	

```
short		/*number of points in contour if NULL PLANE =0 */
float
float
float
float
```

This is followed by 20 shorts, then the contour data x-coordinates given in blocks of 100 shorts, follwed by the contour data y-coordinates given in blocks of 100 shorts.

Universal Contour File

A .ucf file in ASCII format. The header information include image width and height in pixels and the corresponding range in microns, followed by the z-range in microns and the number of sections (levels). The data for each section is then given with the z-value for the section number (level number) followed by the individual contour data points in (x,y,z) format.

Here is what a file would look like:

```
<width>
d
<height>
d
<xrange=>
0.0 f
<yrange=>
0.0 f
<zrange=>
0.0 f
<levels>
d
<level number=>
f
<point_num=>
d
<contour_data=>
f f f
f f f
...
<end of level>
...
<end>
```

ROSS Contour File

The contour file format used by NASA Ames Reconstruction Program (ROSS) is a .asc file in ASCII format. The file has the following structure:

```
data file name:
s
Generated by IGL Trace: d d d
no of: contour entries; planes; groups; max entries;
d d d 3800
section thickness(micrometers); magnification(k);
f f
centroids: x, y, z;
d d d
maximum: x, y; resolution: units/mm;
d d 10
FILE IS ALIGNED
type; no of contours;
d d d
...
plane, obj, type, nrecs, strrec
npts, area, perim;
d d d d d
d 0.0 0.0
d d
...
d d d d d
d 0.0 0.0
d d
...
...
```

AutoCAD DXF File

The DXF file format is a .dxf ASCII file used to represent geometry with many CAD programs. IGL Trace exports a representation of the contours in DXF format as follows. For more details on DXF consult your CAD system reference manual.

```
Study: s generated by IGL Trace: d d d
0
SECTION
2
ENTITIES
0
POLYLINE
8
d
66
1
70
1
0
VERTEX
8
d
10
f
20
f
30
f
...
0
SEQEND
...
0
ENDSEC
0
EOF
```

Nuages Contour File

Contours can be exported to the NUAGES program written by Bernhard Geiger using the Nuages contour file format. This is s .cnt file with all values in ASCII format. The first line gives the number of sections in the series. Followed by the sections in ascending order. Each section is delineated by a lines indicating the number of contours and the z position of the section. Then the contours are given as a series of (x,y) points delineated by curly brackets. For example:

```
S d
v d z f
{
f f
f f
...
}
{
f f
...
}
...
v d z f
{
f f
...
}
...
...
```

2D Raw ASCII Contour File

This simple ASCII format is used to represent named contours of a single section. It can be produced by Exporting the contours from the current section. This same format is used for importing contours into a section, so that contours can be used from other studies or from other sources of data.

In this format, the contours are represented as a series of (x,y) coordinates, given in image coordinates. The values are integers with (0,0) being the upper-left corner of the image as it is displayed on the screen. Unlike other contour file formats, no scaling or modification of the contour data is made during raw export. Each contour is preceded by a name of up to 8 alphanumeric characters on a new line. Then the contour points are given as the series of (x,y) points, each point on a separate line. When there is only one contour, the contour name is optional, otherwise the name strings are necessary to delineate individual contours.

```
s
d d
d d
d d
d d
...
s
d d
d d
d d
...
```

2D HTML Image Map

This format is used for creating WWW pages based on HTML image maps of contour data. It can be produced by Exporting the contours from the current section. The contours are represented as a series of (x,y) coordinates, given in image coordinates. The values are integers with (0,0) being the upper-left corner of the image as it is displayed on the screen. No scaling or modification of the contour data is made during this export.

A map is created with the name of the study. Each contour is output as an area within the map. All contours are represented as poly shapes. The coords are the contours given as the series of (x,y) points. The corresponding href is the contour name with .htm suffix appended.

```
<map name="studynm">
 <area shape=poly coords="0,0,100,0,100,100,0,100,"
   href="contour1.htm">
<area shape=poly coords="10,10,20,20,0,10,"
   href="contour2.htm">
...
</map>
```