Surfaces, Statistics and Contouring

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Topics to be covered

• Automatic isosurface creation
  • Understanding the thresholding options
  • Using the filter tools to isolate your objects
  • Split touching objects
• Manual surface creation
  • Contour tracing
  • Marching cubes/magic wand
• Editing
• Statistics Export
Intensity Threshold Surface as a Wire Frame
• Press the surfaces button to initiate the wizard

• Choose the algorithm settings and click next

• If you want to skip the automatic creation and immediately work manually press the edit button.
Smoothing can result in the loss of small objects/details, so reduce the value or disable if the dataset contains very small features.

- Smoothing applies a Gaussian filter to the data set, most often to reduce noise.

- Use background subtraction, when a single absolute threshold will not segment all objects.
Absolute threshold vs Background subtraction (Local contrast)

- Absolute threshold takes only intensity into account.
- Local contrast searches for local maxima of a given size.
Set the intensity threshold.

New gray surface will be displayed, and dynamically updated as the threshold is changed.

Tip: To change the colour of the preview, or to use a different visualization instead of an Isosurface preview, switch to the Settings tab.
If a large number of triangles are created, rather than display them, to preserve interactivity Imaris will automatically show center points of each surface object, rather than the object itself.

Go to “Settings” and switch the Style from “Center Point” to “Surface” and the newly created surfaces will be displayed.
Step 4 allows pre-filtering of created surface objects. Filters based on high or low values, automatically or manually selected from one or more available measurements.

To finish click the double green arrow.
Split Touching Objects

Sometimes you will have the problem of fusing surfaces, when objects come close to each other.

Check “Split Touching Objects” to use seed points to split these objects.

Hint: Automatic Split Touching Objects is most effective when all the objects within the ROI(s) are of a similar size, and are not too elongated.
Split Touching Objects

- Enter a value slightly close to average size of the objects.
  - If you set it too large, you will miss some of the smallest objects;
  - too small, and you will get multiple seeds in one object

- A 3D “watershed” segmentation is applied, where each seed point represents a “catchment basin”
Filtering the seed points

- Filter the seed points to represent actual number of objects.
- Multiple filters can be combined
Creating Surface manually via Contour tracing

- Visualization & Measurement of structures which cannot be easily segmented from background or neighboring structures

- Draw 3D Regions of Interest to get Statistics for those regions or for masking part of one or more channels
To access the Manual Surfaces dialog, choose Surfaces, then hit:

“Skip automatic creation, go to manual editing”
Manual Surfaces: Mode Tab

- Choose the Contour Tab
- Select the Drawing Board in the Board menu, or by Right-Click onto the slicer
- Select the visibility of the contours
- Copy a contour and paste it on another slice
- Move the slice position. Lock Slicer if needed.

8 TIP: use Keyboard arrows ↑ to move up or ↓ to move down one slice
Clicking within the image inserts a point, a so-called vertex. You can now define subsequent vertices by clicking with the left mouse button. Successive points will be connected with straight-line segments.

**Time**
Click once on the contour and then move the mouse along the contour. The number of vertices depends on the time interval (ms), specified in the corresponding field. The interval can be changed by manually specifying the required value. This drawing style allows you to draw a structure that contains fine details.

**Distance**
Click once on the contour and then simply move the mouse along the contour. The number of vertices depends on the distance interval (um), which is specified in the corresponding field. To change the interval, specify the required value in the object properties area. This drawing style is recommended if the structure is smooth.

**Circle**
The Circle drawing tool inserts a circular contour line. The Circle tool always draws a contour line from the center; that is, the place you initially click will be the center point of the contour line. To change the circle radius specify the required value in the object properties area. In the parameters you can also specify the number of contour vertices to be inserted. To create the contour line, move the mouse pointer over the orthogonal slicer plane. A preview outline of the circle is then displayed. To create a contour line, click with the left mouse button on the desired position.
The Isoline drawing tool generates contours at a fixed iso-value so the geometrical features of the contour line takes into account the image's intensity values. Connecting the points with a constant intensity value forms the contour line.

The Isoline drawing mode selects the maximum intensity iso-value under the mouse pointer and creates a contour line by tracing points of uniform intensity. Clicking with the left mouse button on the orthogonal slicer plane selects the largest connected area that contains the all voxel with the same intensity values. The current iso-value under the mouse pointer is displayed in the parameter field Intensity and is updated automatically as you move the mouse. To create a contour line, click with the left mouse button on the desired position.
Isoline

Reduce density to numerical field %
Defines the density of inserted vertex in relation to the data set.
A density of 100% inserts a contour vertex at each pixel of the data set.
A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

Intensity
Displays the value of the voxel intensity under the mouse pointer.
Magic Wand:

The Magic wand tool creates a contour line by selecting the largest connected area that contains the maximum intensity voxels and all voxels within a user-defined tolerance range. The Tolerance range defines the tool's sensitivity; the higher value you set, the wider image area that is selected. The Tolerance range can be specified in a box in the Parameter panel.

Move the mouse pointer over the orthogonal slicer plane and a preview of the contour line is displayed. The Intensity value in the Parameter panel is updated as the mouse pointer is moved across over the orthogonal slicer plane. To create a contour line, click with the left mouse button on the desired position. The Intensity with the corresponding Tolerance value is stored in the parameter section of the label field. This makes it possible to easily correct a selection at a later time. It is possible to modify the range even after the seed voxel has been selected.
Reduce density to numerical field %
Defines the density of the inserted vertex in relation to the data set.
A density of 100% inserts contour vertex at each pixel of the data set.
A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

Tolerance
Defines the range of intensity limits in percentages.
The tolerance 0% selects pixels with exactly the same intensity value as the point under the mouse pointer. The tolerance value of 100% selects all intensities within the intensity range of the data set. Values in between are interpolated linearly. The tolerance can also be changed incrementally by scrolling the mouse wheel.

Intensity
Displays the value of the voxel intensity under the mouse pointer and the tolerance range.

Detect holes box
This option enables you to create a contour line that contains holes of selected areas. If the Detects holes box is selected the line is generated around holes, outlining the hole. If the box in un-checked, the outer line of the selected region is drawn and the holes are not visible.
AutoFit (= Snakes algorithm), also called active contours, is a method for delineating an object outline from a possibly noisy 2D image.

- Draw a rough initial contour line around the object you would like to segment.
- Run the snakes algorithm with Perform Autofit button to retrieve a contour line which tightly fits the object boundary and looks more regular and smooth.
Other ways of defining Surfaces manually

1) Add New Surface Object
2) Cancel Creation Wizard
Other ways of defining Surfaces manually

- Select Draw mode to Add Surfaces
- Set Autodepth 3D Cursor Behavior
- Choose Segmentation Method
Manually Defined Surfaces: Marching Cubes

Optimized for objects with a complex rough surface.

It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold from the intensity histogram within the bounding box).

The selection box should enclose the object, and approximately be centered. The object created is bounded by the selection cursor.
Manually Defined Surfaces: Magic Wand

Optimized for single, complex objects, which are located over a larger region of the image, but not touching other objects.

The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects.

The threshold is selected from the cursor center, then a closed surface is created by expanding in 3D along the selected intensity contour. Follows the entire object edge, not restricted to the size of the cursor box.
Editing of Surfaces

- Select Edit mode to cut Surfaces or process the selection
- Shift – Left Click to position cutting plane, then cut the surface or clear the scissors
- Process the selected object
- Use Merge to combine surfaces
- Use Mask to create channel from Surface
Change the Pointer mode to “Select” then use “shift”+”Left-Click” to select a vertical line on your surface.

Click “Cut” from the Scissors Properties to split the surfaces.
Filtering Objects for Classification

- Use Add/Delete to change the currently applied filters
- Use the “Filter” drop down list to select the measurements for classification
- Use the Histogram to adjust the classification
- “Duplicate Selection” makes a new copy of currently selected objects
• Go to the statistics tab in the Properties of selected Object

• Check overall, detailed or selection statistics
Use the “Settings” button to select the measurements you are interested in.

Select the:
- “Search by Id” or
- “Duplicate Selection” or
- “Export selection” or
- “Export data for plotting” or
- “Export all statistics”
Color Code Objects by Statistics

Surfaces/Spots/Tracks

- Color Type: Statistics Coded
- Statistics Type: Sphericity
- Colormap: Spectrum
- Min: 0.385, Max: 0.966
- Show Colorbar
- Show Range
- Transparency: 0%
Hands-On Surfaces Exercise 1

Dataset: Sea_Urchin.ims

1. Create Surfaces, Split Touching objects
2. Look for cases where splitting didn’t work
   Use Cut tool to split manually
3. Look for cases where dim objects were missed
   Use Marching Cubes to insert the missed surface(s)
4. Colour-Code Surfaces for Volume
Hands-On Surfaces Exercise 2

Dataset: hypan_004_crop.ims

- Use Contours to mask a region that will allow you to then create a threshold-based Surface from only part of the dataset
- (in this case, a simple region of interest would not give a satisfactory result)
Hands-On Surfaces Exercise 3

Dataset: visu2crop.ims

- Reconstruct some structures of the image manually
- Try out different drawing modes
- Use Ortho Slicers to visualize the reconstructed structures together with the original data

Tip: In many cases, Isoline or AutoFit might be good option