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## Surfaces, Statistics and Contouring

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- Automatic isosurface creation
  - Understanding the thresholding options
  - Using the filter tools to isolate your objects
  - Spilt touching objects
- Manual surface creation
  - Contour tracing
  - Marching cubes/magic wand
- Editing
- Statistics Export



### **Intensity Threshold Surface as a Wire Frame**









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### **Surface Creation Wizard**





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

Smoothing can result in the loss of small objects/details, so reduce the value or disable if the dataset contains very small features

 Use background substraction, when a single absolute threshold will not segment all objects.





#### **Absolute threshold vs** Background substraction (Local contrast)



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



### **Surface Creation Wizard - Threshold**

#### 🤻 Create Color 🍤 Settinas Threshold Threshold (Background Subtraction) M@A 13.4 M@A 84.7 85 Π Split touching Objects (Region Growing) 🔽 🛟 Enable Seed Points Diameter 4.50 um O 3/5

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Set the intensity threshold.

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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.



### **Surface Creation Wizard**





😻 Warning!	R	? 🔀
Considering the total num could lead to a Triangles n	ber of triangles of the surfaces, switching a lack of interactivity or even to a memor node requires more memory and graphic	to Triangles mode now y overflow. s power.
	Do you want to continue?	
Do not show this dialog aga	ain	
		OK Cancel

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.

🔆 Create 🔇 Settings 🍚 Color	🔆 Create 🕚 Settings 🕗 Color
Surfaces Style / Quality	Surfaces Style / Quality
Surface	Surface
Center Point Pixel Width: 5	Center Point Pixel Width: 5
◎ Off	© Off

Go to "Settings" and switch the Style from "Center Point" to "Surface" and the newly created surfaces will be displayed.



### **Surface Creation Wizard**

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🤻 Create	9 Settings	Color		
Classify Surfac	es			
Filters				
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		<b>₩@A</b> 4845		
- N <sub>000</sub> , <sub>N</sub> 00, N <sub>0</sub> 0, N <sup>1</sup> - N <sub>000</sub> , N <sup>1</sup> 1	112 of 222 sel	lected (50%) 4845		
5/5		0000		

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**



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Threshold
Threshold (Background Subtraction)
0 95
Split touching Objects (Region Growing)
🔽 🛟 Enable
Seed Points Diameter 4.50 um
3/5 🕥 🙆 🙆

• 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



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0 200 of 513 selected (39%) 37				
4/5		0	۵ 🕲 🔇	



- Filter the seed points to represent actual number of objects.
- Multiple filters can be combined





 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:

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"Skip automatic creation, go to manual editing"



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#### Click

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.

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#### 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.



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Add/Delete Contour	13
Board Mode Autofit	
Drawing Mode	
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Parameters	
Reduce density to 10.000 %	
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Slice Position	va
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Draw     Create Surface	cre
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Isoline



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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 isovalue 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.



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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.





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Add/Delete Contour
Board Mode Autofit
Drawing Mode
4 4 6 d d
Parameters
Reduce density to 10.000 %
Tolerance: 10.000 %
Intensity: 0.000 +/- 0.000
Detect holes
Channel Selection
All Visible Channel
Specific Channel Channel 1 - cell wall
Slice Position
1 Lock
/ Draw Create Surface

#### 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, than 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.



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#### Magic Wand:

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 Option**



Autofit (= *Snakes* algorithm), also called active contours, is a method for delineating an object outline from a possibly noisy 2D image.

Add/Delete Contour Board Mode Autofit				
Perform	) Autofit			
Presets Shape: Smooth Impact: Weak Advanced	Accurate Strong			
Continuity 1.200 Curvature 1.000 Image Enrg 1.200	Reach 0.750 um Max iter. 10 SSC iter. 2			
Gauss 0.250 um				



- Draw a rough initial contour line around the object you would like to segment.
- Run the snakes algorithm with <u>Perform Autofit</u> button to retrieve a contour line which tightly fits the object boundary and looks more regular and smooth.



### Other ways of defining Surfaces manually

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Add Surface using:

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.







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



### Manual Surfaces: Surface Scissors





Change the Pointer mode to "Select" then use "shift"+"Left-Click" to select a vertical line on your surface.

Cut	
Cut Surface	Clear Scissor
Close Borders	

Click "Cut" from the Scissors Properties to split the surfaces.



### **Filtering Objects for Classification**





- "Sphericity" above 0.725 "Volume" above 191.898 um^3 A 91.898 m^3 🕑 Maa 10.000 m^3 25 of 155 selected (16%) 427 🕙 Duplicate Selection to new Surfaces
- 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



### **Statistics - Overall**



▶ 🗮 🖌 🖉 💽 🌑 🛸				
Overall Detailed Selection				
Variable	Value	Unit		
Number of Disconnected Components per Time Po	pint 187			
Number of Surfaces per Time Point	187			
Total Number of Disconnected Components	187			
Total Number of Surfaces	187			
fotal Number of Triangles	603136			
Total Number of Voxels	326112			
K	🤌 🔕 🛛			

 Go to the statistics tab in the Properties of selected Object

 Check overall, detailed or selection statistics



### **Choosing Statistics and Exporting Data**



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Overall D	etailed Se	ection		
Specific Values				
🗢 Area				
Value	Unit Ca	ategory	ID ^	
103.433	um^2 🖕	Surface	175	
239.689	um^2 🖕	Surface	176	
300.469	um^2 🖕	Surface	177	
123.931	um^2 🖕	Surface	178	
168.378	um^2 🖕	Surface	179	
206.133	um^2 🖕	Surface	280	
189.804	um^2 🖕	Surface	181	
261.391	um^2 💌	Surface	182	
228.418	um^2 🎍	Surface	183	
187.022	um^2 🖌	Surface	184	
238.271	um^2 🖕	Surface	185	
772 077		C		Ł
»				

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 statisitcs"



### **Color Code Objects by Statistics**





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Su	rfaces/Spot	s/Tracks
Color Type Labels Statisti Base Track I Statistics Type Sphericity Colormap: Spectrum Colormap Range Min: 0.385 Max: 0.94 Show Colorbar Show Colorbar Show Range Transparency	CS Coded D Time Mapped Reset 66 Auto Show Title 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

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### 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)









### 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

