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Measurement Points and Spots

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Measuring in Slice View





- Set to "Polygon" to measure non-straight distances
- Left-click on the image to set the points
- Move the slicer if you wish to make 3D measurements
- Distance displayed here



Measuring in Surpass





- View and Edit in interactive 3D
- One or More Pairs
- Polygon Lines

Imaris						
Overall	Detailed	Select	ion			
Distance						~
Value		Unit	Name		ID	-
6.036		um	A-B		1	
5.412		um	B-C		2	
5.424		um	C-D		3	
5.398		um	D-E		4	
6.071		um	E-F		5	
<u>»</u>					€	

- Export All Distances of One Group (and Sum of those Distances) to File
- Combine Multiple Styles within one Scene
- Requires MeasurementPro



Workflow to set Measurement Points





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InMotion

	Camera 🗗	
	Pointer	
	Select	
	🕐 ivavigate	
(属 InMotion	
	Comera Type	
	Orthogonal	
	Perspective 45°	



 InMotion improves ability to see the 3D position of the cursor by rotating the dataset back and forth

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- The cursor is the center of rotation
- While in Select mode, position cursor over object of interest
- The rotation around the cursor helps you to interpret the z position of the cursor



Distance Measurement







- Use different Object types for different specimens and locations you want to reach
- Surface objects to measure from surface to surface, or from center to center.
- Volume to measure from center to center of relatively small objects, or when there is no surface or spot generated.
- Slicer for all other positions especially inside of bigger filled objects









- Go to Intensity Tab
- Select pair of points, or all points for the line profile

Line A-B	
Line B-C	
Line C-D	
Line D-E	
Line E-F	
Line A-B-	C-D-E-F

It is possible to export snapshot of the line profile



Spot Detection



- Efficient, Robust blob/point detector
 - Better at coping with noise and background
 - Works better with inconsistent intensity
 - Better at keeping touching objects separated
 - Compared to Surfaces, fewer parameters, quicker calculation, less work for graphics card, less memory
- Limitations
 - Can't use Spots if you want to measure shape statistics (Sphericity, Ellipticity, axis orientations, etc.)







- "z-stretching", why?
- Imaging limitations low resolution low N.A.
- Real biological feature

Common detection artifacts

- Localization
- Multiple detection
- Intensity quantification precision



Volume Rendered Object

Spherical Spots (artifact)



Ellipsoid Spots (Z adjusted) correct object detection





A tool that more closely matches the shape of the object without loosing the key performance advantages over Surface detection.

- higher detection
 Speed
- lower RAM requirements

Volume Rendered Object



Ellipsoid Spots (correct)



Ellipsoid Spots (Z adjusted) correct tracking





Tracking potential artifact (location)

Correct tracking using ellipsoid spots



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	Spots 1 - Properties
For automatic segmentation of objects, imaris uses creation wizards which guide you through all the steps of the process and facilitate the use of complex functionality	 Scene Light Source 1 Frame Volume Spots 1
 Press the spots button to initiate the wizard 	Create Settings Color Algorithm Skip automatic creation, edit manually Favorite Creation Parameters Default
Choose the algorithm settings	Segment only a Region of Interest Process entire Image finally Different Spot Sizes (Region Growing) Track Spate (over Time)
 If you want to skip the automatic creation and immediately work manually press the edit button. 	





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Favorite Crea	tion Parameters
Default *	• 🕺
Algorithm Se	ttings
Segment of	only a Region of Interest
Process	s entire Image finally
Different S	Spot Sizes (Region Growing)
Track Spo	ts (over Time)
Track Spo	ts (over Time)

- Segment a region of interest to test parameters quickly
 - At the end, same parameters can be applied to entire image
- Different spot sizes are optional
 - Region Growing from Spots has similarities to Split Touching Objects from Surfaces
 - The delta from smallest to largest cannot be too great



Sub Region Processing





🤻 Create	•	Settings	•	Color	
Region of Inte	rest				
Region of Inte	erest 1	l)			
Min:		Max:		Size:	
X: 37	-	222	-	186	*
Y: 46	-	204	-	159	*
Z: 1	-	32	-	32	*
Memory Usage	e: 0.90	3 MB			
Regions of In	terest	aract 1		Add	12
Kegion (of Inte	erest 1		Add	
				Delet	e
Total Memory	Usage	: 0.903 MB			

- Position region of interest (ROI)
 - Pointer must be in Select mode to drag the box or arrows
- Option of applying parameters to multiple ROIs with "Add" button.



Spot Detection works as follows:

- 1. Filter (smooth) the image with the Gaussian or Mexican Hat** filter.
- 2. Spots are located at local maxima of the filtered image.

Source Channel
Channel 2 - chloroplast

Channel 2 - chloroplast

Spot Detection

Estimated XY Diameter: 2.50
um

Detect Ellipsoids
Estimated Z Diameter: 2.50
um

Background Subtraction

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Tip: Use Slice view to take a quick measurement to determine estimated diameter in XY plane (check "Detect Ellipsoids" and determine estimate diameter along z-axis if you would like to detect ellipsoid spot)



**often used technical term for what is called Background Subtraction or Local Contrast in Imaris





Mexican Hat Effect

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- Often the objects in the image do not have the same intensity
- Threshold too high: dim objects will be missed
- Threshold too low: too much background is above the threshold
 - Apply 3D Mexican Hat filter of spot size
- Background is now lower than all the object peaks
- A single threshold height could now include all the objects, and exclude all the background



Spot Detection / Classification



- The initial threshold is intelligently set by Imaris based on your data
- It is calculated from all spots based on kmeans statistical method.
- Increasing or decreasing the threshold value will allow you to include or exclude spots in the image
- As you drag the threshold value, the image will change interactively as spots appear and disappear
- When satisfied with the Spots, click Next.



Filtering Spots for Classification







Create	🝰 Setting:	s 🔮 Color	
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🗹 Different	Spot Sizes (R	egion Growing)	
Track Spo	ots (over Time))	
	Create	🝰 Settings	Color
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	Local Cor	ntrast	
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- Spots in Imaris are very robust object-position-detectors
- The size of regions around spots can be computed as well.

- This can be useful for measuring:
 - Diameter / Volume for each Spot
 - Intensity within each Spot



Region Growing Threshold





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- Region Volume the radius of the spot is calculated from the region volume (meaning the region volume and the spot volume will be equal)
- Region Border the radius of the spot is calculated as the smallest distance from each spot center to the region border





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Rebuilding Spots

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•	Choose the Rebuild tab to go back through the entire creation process again or to go through only part of the creation process

- All previous selections are already in place and can be tweaked
- Provides a useful list of exactly what functions you have used in the creation of the object
- Can be used as an input for ImarisBatch or • as a Favourite setting

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Name		
Spots 1		
Store Location		
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Favorite Creation Pa	arameters	



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Rebuild	
Algorithm 👻	
Rebuild	
Creation Parameters	
[Algorithm] Enable Region Of Interest = true Process Entire Image = true Enable Region Growing = false Enable Tracking = false [Region of Interest] Region1: XYZT from [182 124 1 1] to [228 185 Region2: XYZT from [30 102 1 1] to [84 171 32 [Source Channel] Source Channel Index = 2 Estimated Diameter = 0.670 um Background Subtraction = true [Classify Spots] "Quality" above automatic threshold	
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Editing Spots



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Enable Delay before auto-advancing

- shift and left click to add a spot
- To delete, shift and click on the selected spot
- Center the spot in 3D based on the local intensities
- The diameter of the spot can be adjusted using the scroll button on the mouse, or in the object menu



Filtering Spots for Classification





• Use Add/Delete to change the currently applied filters

Filters are Cumulative/Additive (e.g. Diameter above X and Intensity Mean above Y)

- Use the "Filter" drop down list to select the measurements for classification
- Use the Histogram to adjust the classification based on the measured statistics. Histogram shading corresponds to the highlighted Spots
- Duplicate Selection, copies only the selected objects to a new spots object



Spot Statistics

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Overall	Detailed	Selection	
Specific Values	5		•
🐏 Area			•
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74.988	um^2	✤ Spot	18(
85.098	um^2	✤ Spot	18:
82.995	um^2	✤ Spot	18.
43.538	um^2	✤ Spot	18:
140.691	um^2	🔹 Spot	184
105.671	um^2	🔹 Spot	18!
2.919	um^2	 Spot 	18(
107.859	um^2	🔹 Spot	18.
128.891	um^2	• Spot	18
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- Statistics are based on the voxels that are contained within the spot sphere (the size of the sphere for the spot affects these measurements)
 - Per Spot statistics (in Excel Export):
 - Spot Area, Volume, X,Y,Z position
 - Intensity at Center
 - Max, Min, StdDev, Mean, Median, Sum (per color channel) for all voxels inside spot

PLA

- Diameter XYZ, # of Voxels
- Per 3D image (time point)
 - Number of Spots
 - Average Intensity at Center, Mean Intensity
 - Sum of the Volumes, Areas
- Track statistics





Spots Selection: 3D Scene

- Select spots by left-click in the 3D scene
- Multiple Select spots by ctrl + left-click in the 3D scene (cmd + left click for osx)



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 Selection is displayed in the statistics tab



Spots Selection: Statistics Tab



- Click on a row in the table
- Multiple-select rows in the table with Ctrl or Shift

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			8
Overall	Detailed	Selection	n
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Selection is displayed in the 3D scene





Select Statistics Coded, a Statistics Type and a Colormap. Adjust the Colormap Range accordingly.



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Statistics Type	
* Position X	•
Colormap: Bitplane 3 🔹	Reset
Colormap Range	
Min: 0.125 Max: 153.875	Auto
Show Colorbar	
🗹 Show Range 🚺 Show	Title
Transparency	
Trancha	ronge: 0.94
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Labels



The labels toolbar can be used to label various objects manually, or according to the filter options available for the data type. Labels provide a useful way to tag and identify a subset of objects for further analysis. The label or label group name (including partial string) may also be used in the Imaris search (Arena) to quickly locate any datasets which use that label.

Labels of Selection	Тс
 Default Labels *Cells Nucleus 	bu ur be
Add a label	
+ - Default Labe	is 👻
*Cells Nucleus	Add Label to Remove Labe
👰 Full Screen 🔞	Add a new La Remove Labe Edit Label
	Show Help

To create a new label, you should first create a new label group. To do this, click the + button and enter the name of the new label group (Note this field is highlighted red if unsupported characters are used, or the field is empty). The new label group will now be available from the drop-down menu. If you need to delete a label group, select the label group to be deleted and click the - button.

Add Label to Selected Objects Remove Label from Selected Objects Add a new Label to current Group... Remove Label from current Group... Edit Label... To add a label to the label group, select the label group in the drop-down menu, click the **Add a label** field, or rightclick the **label selection box** (lower box) and enter the names for each label that is to be part of the selected label group. The color of the new label is assigned at random. This can be changed using the **Edit Label** option.



Label Color Coding

or right+click for the menu

by double-clicking on the label

Labeled objects can get displayed

according to their label color

Labels can be added either with the + sign

Selected objects can get assigned a label

are displayed in the base color.

Note that if there is more than one

label associated with an object, the

object will be displayed as a

combined color. Non-labelled objects

Add Label to Selected Objects Remove Label from Selected Objects Add a new Label to current Group... Remove Label from current Group... Edit Label...

Show Help

Labels are stored for the use in other Imaris instances



Base Track ID Time Mappe	Labels	Statistics Coded
Label	Base	Track ID Time Mapped
 Default Labels Cells Nucleus 	Label	
		Cells
Only show labelled objects		Nucleus
	Only shov Transparency	Nucleus v labelled objects



Full Screen

Labels of Selection

Add a label

*Cells

Nucleus

Default Labels

*Cells

Nucleus

Default Labels

- Dataset: Particle-1.ims
- Exercises:
 - 1. Compare intensity profiles of a few nearby bright & dim particles
 - 2. Count number of particles using Spots detection (use Slice View to estimate diameter)
 - 3. Statistically code Spots color based on median intensity within each sphere





Dataset: resam-crop.ims

- 1. Count cells by creating spots. Detect multiple spot sizes.
- 2. Filter spots for Volume smaller than $250 \ \mu m^3$
- 3. Colour code the new subset of spots for Volume



