

Media Cybernetics Product Note

Creating Volume Measurements in 3D Constructor[®]

Introduction

Three-dimensional volume construction is a technique being used more often in microscopy. As researchers seek more information from image volumes, the need grows to understand more than just the structure itself. Quantitative measurements such as volume and distance are required to further reveal details about the volume and its related substructures.

Hardware components are readily available and computer processing power is affordable and easily obtainable to perform quantitative volume analyses. 3D Constructor version 5.0 and higher provide a simple means for determining volume measurements from just about any three-dimensional image stack. It also provides measurements for colocalized regions within the volume. Many different measurements are available.

Applications and Examples

3D Constructor is a plug-in module designed for use within Image-Pro[®] Plus. It contains functions for creating both distance and volume measurements. A wide variety of image stack formats may be imported from Image-Pro Plus including popular confocal formats and commonly used image stack formats such as TIFF (Table 1).

- | | |
|----------------------------|---------------------------|
| • Nikon C1 (ICS) | • AutoQuant (DEB, AVZ) |
| • TIFF | • Universal Imaging (STK) |
| • JPEG | • BioRad (PIC) |
| • NIH (RAW) | • Leica (LEI) |
| • Molecular Dynamics (GEL) | • Zeiss (LSM) |
| • Olympus Fluoview (TIF) | • Windows Movie (AVI) |

Table 1. Popular Image File Formats Supported by 3D Constructor

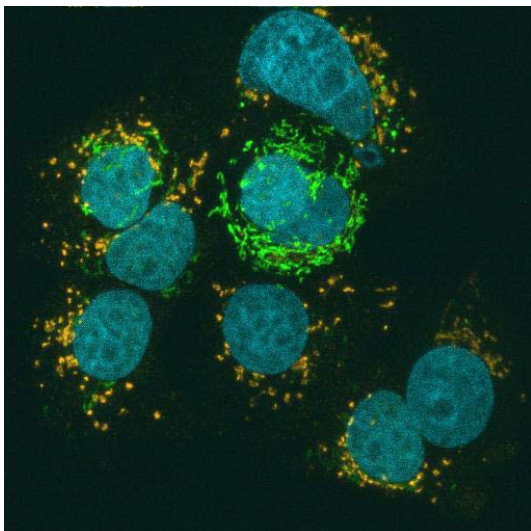
3D Constructor can measure several volume attributes from the imported image stacks. These attributes include but are not limited to volume, surface area, width, height, sphericity and distance between subvolumes. Measurement information can be obtained for objects that have been fluorescently labeled or acquired in brightfield environments. If a volume is composed of more than one fluorophore it is possible to obtain measurements for subvolumes labeled with individual fluorophores and colocalized regions for specific fluorescent pairs. Editing tools are available to filter volumes based on their measured attributes and to split adjoining objects.

How Does it Work?

To obtain volume information, 3D Constructor uses interactive thresholding and surface modeling methods to create the volumes for the objects of interest. 3D Constructor takes threshold information input by the user and creates a ‘surface value’ based on the objects’ color (if it is a color image volume) and intensity. The surface value is created by assigning the threshold to a level the user feels best represents the objects surface. The surface itself is represented by a series of interconnected triangles that approximate the previously established surface values. These triangles can be instructed to follow surface contours with varying degrees of accuracy. To aid in visualization the surfaces are displayed in solid colors and are used to determine volumetric and surface area attributes.

Which Volume Attributes Can I Measure?

Many measurements related to volume are available, as are distance measurements between volumes. For a more in-depth look at distance measurements, please see our product note, “Creating Spatial Measurements in 3D Constructor”.



MCF7 Breast Carcinoma Cells. LAMP1 (Alexa 568- Red), P32 (Alexa 488- Green), Nuclei (DAPI- Blue). Courtesy of Ed Monosov- Burnham Institute, La Jolla, CA.

The following volume measurements can be created in 3D Constructor:

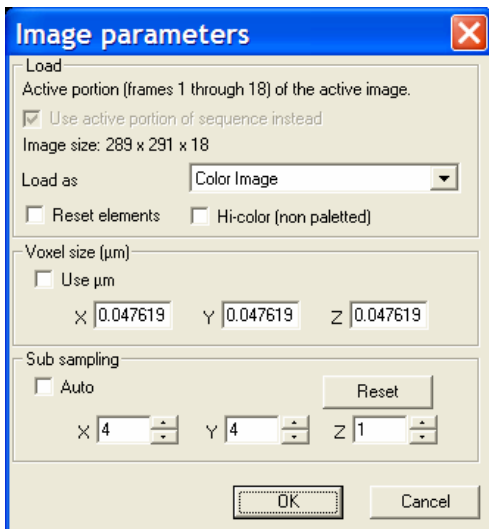
- | | |
|-------------------|---------------------------------|
| • Volume | • Sphericity |
| • Surface Area | • Mean Density |
| • Width | • Integrated Optical Density |
| • Height | • Standard Deviation of Density |
| • Depth | • Maximum Radius of Volume |
| • Center X | • Minimum Radius of Volume |
| • Center Y | • Radius Ratio |
| • Center Z | • Object Class |
| • Box Volume | • Maximum Feret Diameter |
| • Box Ratio | • Minimum Feret Diameter |
| • Volume Fraction | • Feret Ratio |
| • Diameter | |

Additional measurements to determine distances between the closest edges of two volumes or their geographic centers are also available.

Implementation

Advanced|3D Constructor...

The 3D Constructor Image Parameters menu appears:



The 3D Constructor Image Parameters Menu

The ‘Load’ grouping contains information about the active image stack. This group will be updated depending upon which image is active.

- If no image planes have been removed from the active stack, ‘Active portion (frames x through y) of the active image’ will be the selected loading function.
- If image planes have been removed, or if a group of planes has been extracted from the stack, you can click the ‘Use active portion of sequence instead’ check box. This function

will apply if you have selected a sub-sampled portion of the image stack.

- ‘Load as’ allows you to load the entire image or selected color components of an image stack, including multiple channels and colocalization pairs if it is a fluorescent image. The following options are available:
 - Grayscale image
 - Color Image
 - Red Channel
 - Green Channel
 - Blue Channel
- Click ‘Reset elements’ to remove any settings used from previous experiments.
- Click ‘Hi-color (non-paletted)’ to display the image in an enriched color format. Note that by doing so, you will increase the demands on the graphics adapter’s memory.

The ‘Voxel size (um)’ grouping contains calibrated units carried from the image volume (if any) into the three-dimensional image stack. Values will be updated into the X, Y and Z boxes respectively.

- Click ‘Use µm’ if the image is not calibrated and you know the calibrated values in microns for X, Y and Z. Input each known value into the boxes.

The ‘Sub sampling’ group allows you to simplify the rendering of the image stack. By sub-sampling the stack, volume rotations will become more fluid. Raw volumes can become quite large with respect to the amount of available video memory, and oftentimes it is not necessary to render each pixel in each image plane. By sub-sampling the available data, you are able to freely rotate samples while maintaining the overall detail within the image.

- You may type in sub-sampling values if you are familiar with your image stacks, and have modeled similar types of stacks. You can also type in values as a means for experimenting with which settings work best for your stacks. If the outcome is not to your liking, you can always click ‘Reset’ to change the settings back to default levels.
- Click ‘Auto’ if you would like 3D Constructor to determine sub-sampling values for you. If the outcome is not to your liking, you can

always to change the settings by deselecting 'Auto' and typing in different values.

After the sub-sampling has been determined and the image stack displayed as a volume in 3D Constructor, you can now get to work finding volumes.

Creating Volumes

- From the 3D Constructor Options menu, click '↓' and select Volume Measurements (Fig 1).
- Click 'Add'.

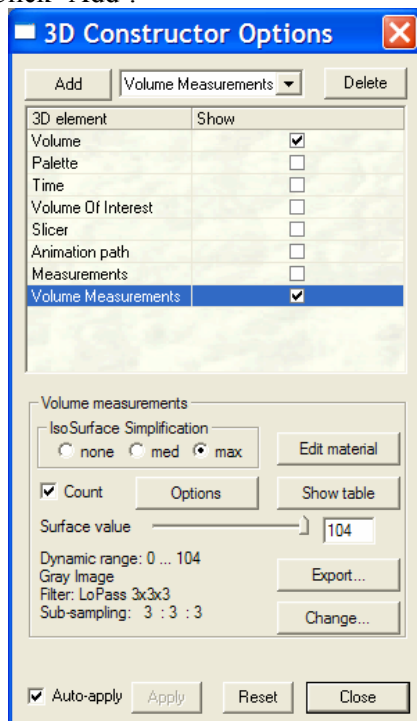
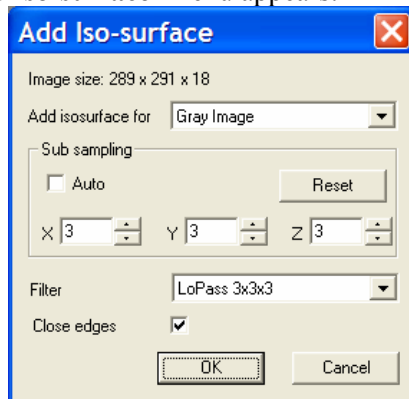


Figure 1. Adding Volume Measurements

The 'Add Iso-surface' menu appears:



- Select the channel for which you would like to add the iso-surface. The following options are available:

- Gray Image
- Red Channel
- Green Channel
- Blue Channel
- Red-Green Colocalization
- Red-Blue Colocalization
- Blue-Green Colocalization

- Select a sub-sampling level for which to create the iso-surface. You may ask 3D Constructor to do this by clicking 'Auto'.
- Select an enhancement filter to apply to the image stack. You can select from several levels of LoPass (smoothing low, medium and high), Gaussian (enhanced smoothing low, medium or high) or None.

A Word About Adding Iso-surfaces

- Subsampling will greatly simplify the surface and improve rotation. But be careful not to oversample and remove surface features!
- Iso-surface simplification also helps improve the surface rendering and image rotation.
- Applying filters are quite useful when attempting to suppress noise in the image stack or when trying to improve contrast between adjacent areas. You can always undo a filter, so don't be afraid to experiment.
- Surfaces may be created for each color channel in the image.

- Click 'Close Edges' to seal the edges of any portion of the image stack that may be touching the volume extents.
- Click 'OK'.
- In '3D Constructor Options', an Iso-surface Simplification group and 'Surface Value' slider appear (Figure 1).
 - Select a simplification level. The more complex a surface, the more you may want to simplify it.
 - Adjust the Surface Value slider to create the iso-surface. A dynamic preview appears, revealing the rendered surfaces (Fig 1).
- If all you are interested in is a surface rendering for a given channel, deselect the 'Count' checkbox in the Volume Measurements group. Surfaces will be created for the selected color channel or colocalization pair without any measurements being generated.

- To count objects that have a rendered surface, select the 'Count' checkbox in the Volume Measurements submenu.
 - Select 'Show Table' to see the Volume Measurements Data Table (Fig 3).
 - Select 'Options' to add/remove values in the Volume Measurement table. Click the checkbox next to the measurement to activate it; uncheck the box to deactivate it (Fig 4).
 - To view a measurement on the image, click and drag the measurement of interest to the top of the Volume Measurement Options list. In the Labels group choose 'Show' and select 'First Measurement' from the drop-down list.

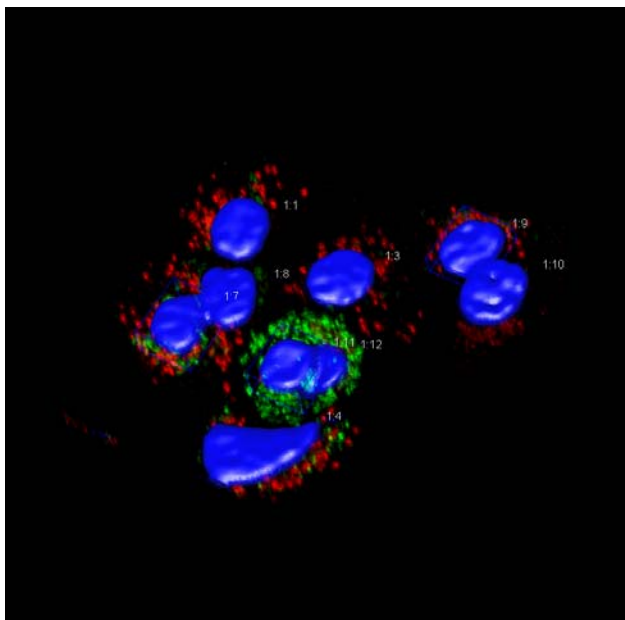


Figure 2. Nuclear volumes (blue) created through the use of the Surface Value slider. Blue boxes indicate selected volumes in Figure 3.

#	Name	Volume	Surface area	Density (mean)
1	1:1	674.5445	457.6883	42.8037
2	1:3	602.4449	451.2314	38.8568
3	1:4	1396.0201	863.3344	46.4775
4	1:7	718.0426	457.2794	48.8721
5	1:8	727.5314	532.5827	42.2635
6	1:9	546.1724	418.4050	38.3138
7	1:10	768.3872	573.5599	36.6625
8	1:11	692.7673	455.0779	57.0714
9	1:12	516.7738	427.6617	44.9718

Figure 3. The Volume Measurements Data Table

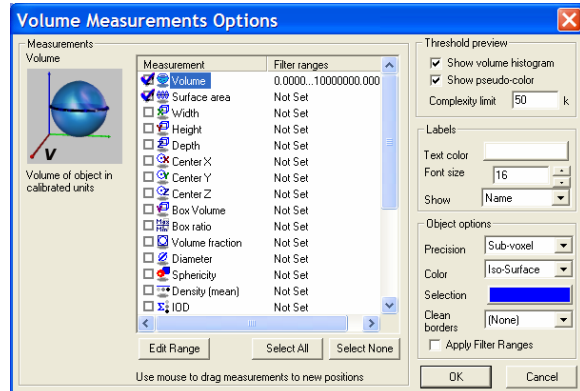


Figure 4. The Volume Measurements Options Menu

See Also

Media Cybernetics Product Note- "Color Composite".

Media Cybernetics Product Note- "Extended Depth of Field".

Media Cybernetics Product Note- "Creating Spatial Measurements in 3D Constructor"

Media Cybernetics White Paper- "Extended Depth of Field".

Media Cybernetics White Paper- "Visualizing the Accumulation of CD3 at the Immune Synapse in Activated T-Cells Using 3D Constructor"

Media Cybernetics White Paper- "The Basics of Using 3D Constructor"

Media Cybernetics Solution #1275- 3D Colocalization.

Related Products

Image-Pro® Discovery 3D

Image-Pro® Plus MDA (Multi-Dimensional Analysis)

AFA™- Advanced Fluorescence Acquisition Software

Scope-Pro® - Microscope Automation Software

SharpStack® - Deconvolution and Deblurring Software

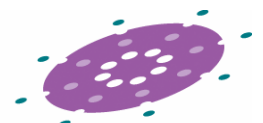
Media Cybernetics Automation Kits

Media Cybernetics Camera Kits

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