

# Media Cybernetics Product Note

## Creating Spatial 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 spatial analyses involving more than three-dimensions. 3D Constructor versions 5.0 and higher provide a simple means for determining these measurements from just about any three-dimensional image stack. A multitude of spatial measurements are available.

### Applications and Examples

3D Constructor is a plug-in module designed for use within Image-Pro® Plus version 4.5.1 or later. It contains functions for creating distance, area and volume measurements. A wide variety of image formats (Table 1) may be imported including popular confocal formats and commonly used image formats such as TIFF.

- |                            |                           |
|----------------------------|---------------------------|
| • 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 spatial attributes from the imported image stacks. These attributes include but are not limited to straight line distances between points, a ‘polyline’ linking several points along a surface, angles surface areas and distances between sub-volumes. Measurement information can be obtained for objects that have been fluorescently labeled or acquired in brightfield environments. Editing tools are available to refine and observe characteristics in the measured data.

### How Does it Work?

3D Constructor uses voxel information provided from the image stack to create spatial measurements in three dimensions. Voxels are three-dimensional picture elements containing not only intensity information, but spatial information as well. By knowing the x, y and z dimensions of the voxels that create an image volume, spatial measurements such as distance, angle and cross-sectional area can be determined.

### What Sort of Spatial Measurements Can I Make?

Many measurements related to distance and areas are available. Additional methods are available in 3D Constructor to create volume measurements. For a more in-depth look at creating volumes, please see our product note, “Creating Volume Measurements in 3D Constructor”.

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

- |            |           |
|------------|-----------|
| • Length   | • Start X |
| • Angle    | • Start Y |
| • Area     | • Start Z |
| • Center X | • End X   |
| • Center Y | • End Y   |
| • Center Z | • End Z   |

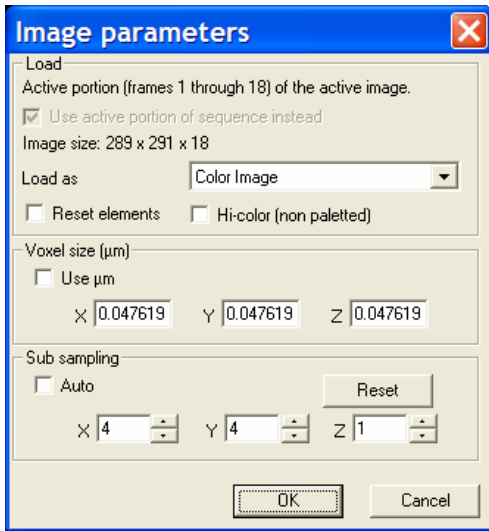
Additional measurements to determine distances between the closest edges of two volumes or their geographic centers are also available.

Spatial measurements may be reported in either the calibrated unit or pixel values.

### 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  $\mu\text{m}$ ’ 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 creating spatial measurements.

### **Creating Measurements**

- Highlight ‘Manual Measurements’ in the 3D Constructor Options Menu (Fig 1).

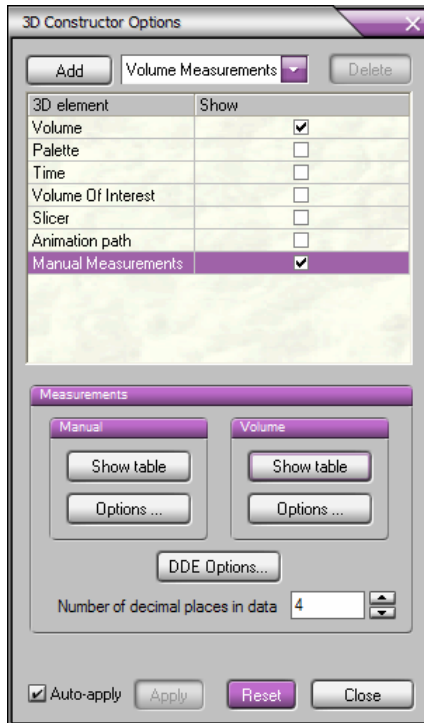
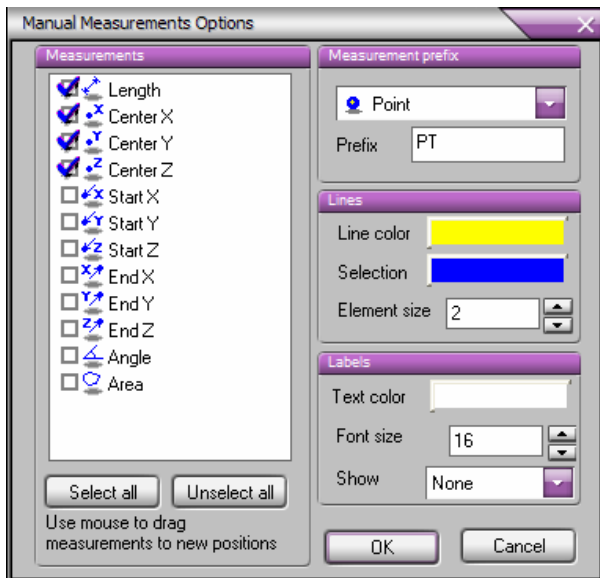


Figure 1. The Manual Measurements Group

In the Measurements Group click Manual| ‘Options...’  
The Manual Measurements Options group appears:

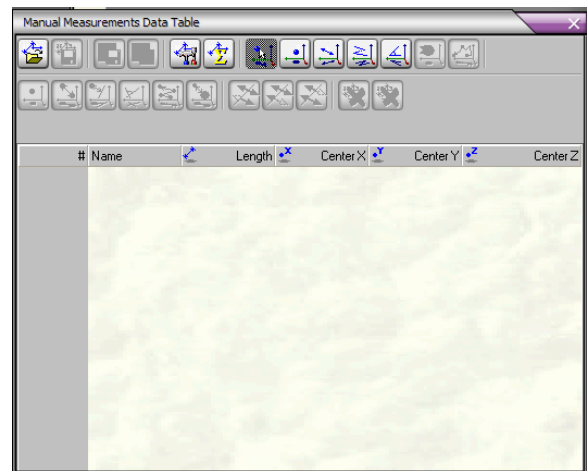


The Manual Measurements Options Menu

**A Word About Creating Spatial Measurements**

- When creating point-to-point measurements be sure to rotate the volume to accurately place the points.
- Points, lines and angles may only be created on points on or within the volume.
- Lines, polylines and angles may all be created on volumes containing an isosurface.
- To create cross sectional area measurements and polylines connecting two points you must first create an isosurface.
- Combinations of measurements may all be created within the same image volume.



- Select which measurements you would like to create by clicking the checkbox next to the measurement.
- Click ‘OK’ to return to the 3D Constructor Options group and the Manual Measurements group.
- Click ‘Show Table’ to show the Manual Measurements Data table:





The Manual Measurements Data Table

Two different types of measurements are available-  
New Measurements and Derived Measurements.

## Creating New Measurements

-  Click the ‘Point’ tool button to determine the x, y and z coordinates for each point you place in the volume. Click the left mouse button to place each point. (Fig. 2).
-  Click the ‘Line’ tool button to draw a line between two positions within the volume (Fig 2).

-  Click the 'Polyline' tool button to draw a line that 'follows the surface'. Click the left mouse button at each of the locations the line should be placed. Click the right mouse button to anchor the line (Fig 2).
-  Click the 'Angle' tool to draw an angle within the image volume. Click the left mouse button to select the first position, again to select the angle's origin and a third time to define the angle (Fig 2).

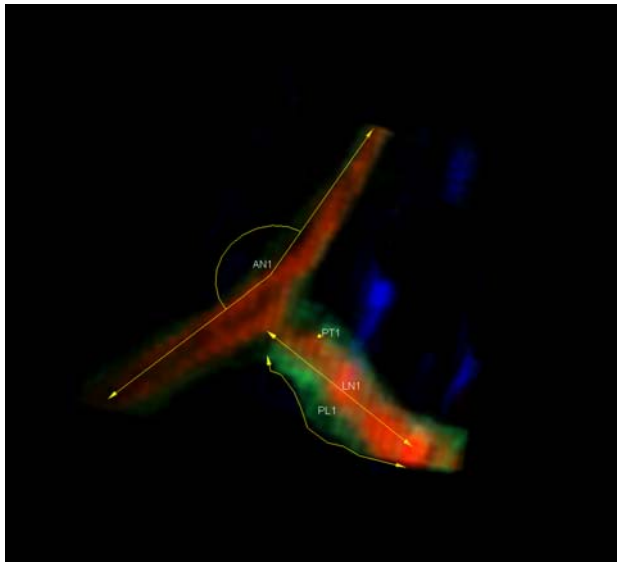






Figure 2. Creating new measurements within a volume. Straight lines (LN1), Poly-lines (PL1), Points (PT1) and Angles (AN1).

## Creating Derived Measurements

Derived measurements are only active when two or more features are available in the Manual Measurements Data Table.

-  Click the 'Measure Distance Between Centers' tool to measure the distance between two selected values in the Data Table (Fig 3).
-  Click the 'Measure Perpendicular Distance Between Point and Line' to measure the distance between a selected point and line in the Data Table (Fig 3).
-  Click the 'Measure Angle Between Lines' tool to measure the angle between two selected lines in the Data Table (Fig 3).
-  Click the 'Create Polyline Using Selected Measurements' tool to create a poly-line connecting two or more selected points, lines

or combinations of points and lines in the Data Table (Fig 3).

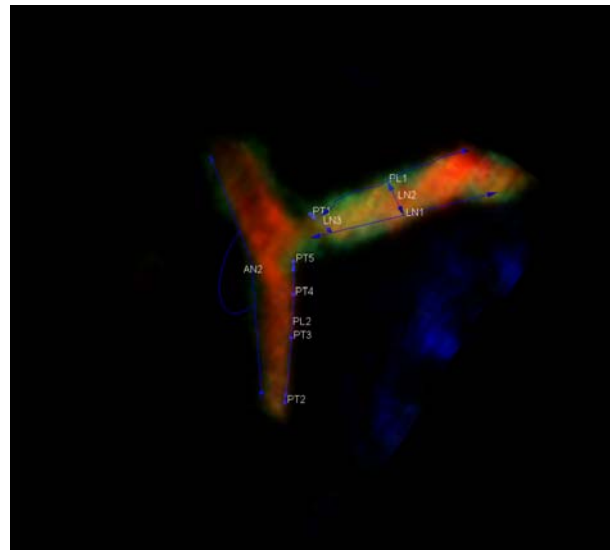




Figure 3. Creating derived measurements within a volume. Measure Distance Between Centers (LN2), Measure Perpendicular Distance Between Point and Line (LN3), Measure Angle Between Lines (AN2), Create Polyline Using Selected Measurements (PT2, PT3, PT4, PT5). Note that line colors may be changed to increase visibility within the volume.

-  Click the 'Area' tool to determine the cross-sectional area of an object within the volume. Click the first point to define the start of the line. Click a second point to draw a line through that point and connect it with the first point (Fig 4).
-  Click the 'Polyline Connecting Two Points' tool to connect two points on a surface. You can select two points from the measurement list or create the points by clicking on the appropriate positions on the volume surface (Fig 4).

**Note:** Prior to using the 'Area', 'Polyline Connecting Two Points' and 'Distance Between a Point and Surface of a Volume' tools an isosurface must be created. You can refer to the Product Note 'Creating Three Dimensional Volumes' for a description of how to complete this task.

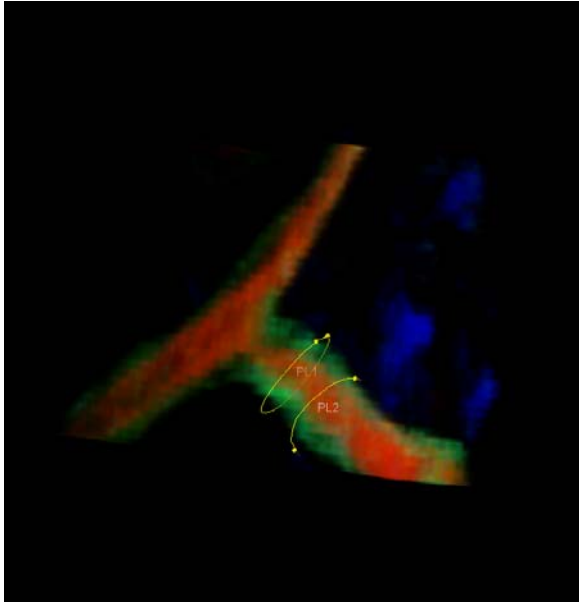



Figure 4. Creating derived measurements within a volume. Area (PL1) and Polyline Connecting Two Points (PL2). Although an isosurface is required to create these two measurements, it has been turned off to show the labels and placement of the polylines.

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 Click the ‘Distance Between a Point and Surface of a Volume’ to measure the distance between a point selected from the Data Table and a volume selected from the Volume Data Table (Fig 5).

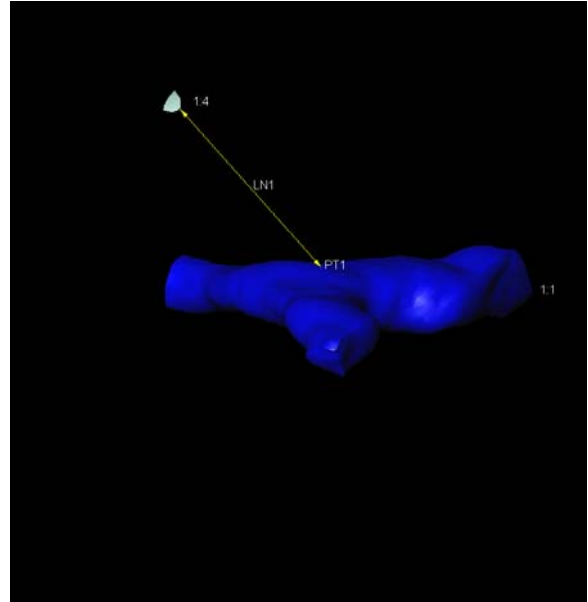


Figure 5. Creating distance measurements (LN1) between a point (PT1) and the surface of a volume (1:4).

### See Also

Media Cybernetics Product Note- “Color Composite”.  
 Media Cybernetics Product Note- “Extended Depth of Field”.  
 Media Cybernetics White Paper- “Extended Depth of Field”.  
 Media Cybernetics White Paper- “Deconvolution and Deblurring Techniques”.  
 Media Cybernetics Solution #1275- 3D Colocalization.

### Related Products

Image-Pro Discovery™ 3D  
 AFA™- Advanced Fluorescence Acquisition Software  
 Scope-Pro® - Microscope Automation Software  
 SharpStack® - Deconvolution and Deblurring Software  
 Media Cybernetics Automation Kits  
 Media Cybernetics Camera Kits

### How to Order

For more information about 3D Constructor, and to locate a Media Cybernetics reseller in your area, visit our website at [www.mediacy.com](http://www.mediacy.com).

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