Introduction

SimplePCI **V**isualization **M**ulti-**D**imensional Viewer (**VIS-MD**) is Compix's new fast and interactive 3D viewer. VIS includes user selectable options to render, measure, and animate multi-dimensional images acquired with the SimplePCI Automated Image Capture Module. **VIS-MD** renders images using optimized software and hardware acceleration.

The modules support the following features:
- Views: 3D View, Channel View, Orthogonal Slices, Colocalized view
- Multi-site XYZ timepoints
- Projections: Maximum, Minimum, Sum, Alpha Blend
- Export 3D view as AVI, CXD, or Tiff
- Visible Volume Measurement
- Interactively Measure Distance Between Two Points
- Visualize Colocalized Points
- Arbitrary Clip Plane

Expand the functionality of **VIS-MD** by adding the following optional modules:
- **AIC,** automated control and image acquisition
- **DIA**, dynamically measures intensity changes on- or off-line
- **IPA**, develops icon-driven work files for automatic image analysis and processing
- **IPA-MTA**, track and analyze moving objects
- **QFA-FRET**, accurate FRET measurements and cross talk correction

Getting Started

Quick Start contains examples of how to utilize **VIS-MD**. For further assistance, refer to the online help, manual, or visit support at http://www.cimaging.net, for access to the latest **How to's** and frequently asked questions. Additional support is available via e-mail: support@cimaging.net or Tel: 412-741-7920.

Example guides:

Introduction to VIS

1. Open a data document. **File** > **Open** > **Data Document** > Select File > **Open** (fig.1).

fig.1

2. Click on the **Multi-dimensional Viewer** icon ⬚ ▾ on the SimplePCI toolbar. The application starts transferring the Data into the 3D viewer.

3. By default, a maximum projection of the 3D stack is displayed.

4. Click and drag to interact with the rendered sample. Below the image is the Rotation slider. Click on the X, Y or Z to rotate along the selected axis using the slider or the playback buttons. Click on **Reset** to clear the X, Y, Z settings, and return to the default values of 0.

5. **Zoom In/Out** by using your mouse scroll wheel.

fig.2

6. Changing Color channels. Click a color channel and select a color or wavelength from the window.
Note: The list of color or wavelength can be customized in SimplePCI.

7. Enter **X,Y, Z Spatial Calibration** for your acquisition device. X, Y is the pixels size value determined in the calibration exercise in SimplePCI. Z is the step size used between slices during the acquisition. These values are automatically de-tected from the data set.
Check Correct Aspect Ratio to use the calibrated values.
Note: if your image doesn't look proportionally correct, double check your spatial calibration values.

8. Enable Bounding Box to rotate and cut through the sample's optical axis. Go to **View** > Select **Display Bounding Box** (fig.3). Click + drag on the white ball to cut through the sample. Click + drag on the box to rotate. Click **Reset Volume** to clear the current settings, and set the default values (fig.2).

fig.3

9. Display XYZ Axis. Go to **View** > Select **Display Axis**. The X axis is red, the Y axis is green and the Z axis is blue.

10. Changing 3D projection view. Go to **View** > select **Alpha Blend** or any of the available 3D projection Views (fig.3). **Note**: if the Alpha blend appears too dark go to the **Multidi-mensional Viewer Properties**, available via the down arrow, and increase the threshold values (fig.4).

11. Export 3D data as a 3D Animation AVI file. Go to **File** > **Export Movie** > Select **AVI** as file type > **Export**.
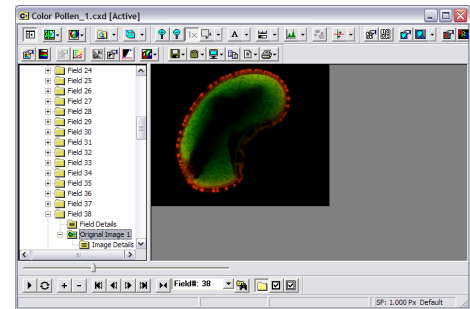
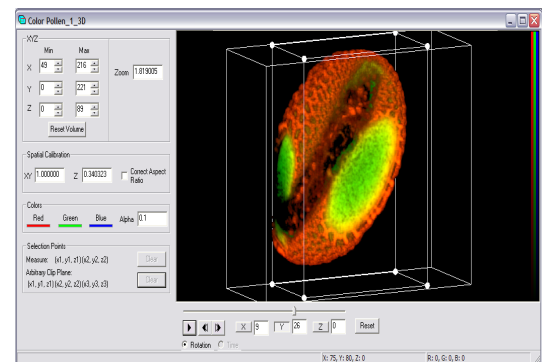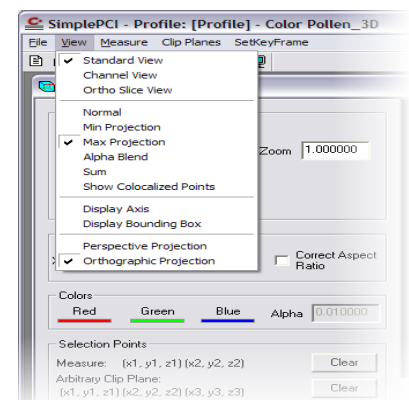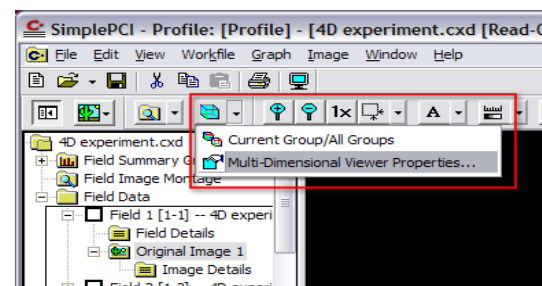12. Close the viewer to return to the **Data Document**.

fig.4

Measuring Distance Between Two Points

1. Open a data document. **File** > **Open** > **Data Document**> Select **File** > **Open** (fig.1).

2. Click on the "**Multi-dimensional Viewer**" icon on the SimplePCI toolbar. The application starts transferring the Data into the 3D viewer.

3. Click and drag to rotate the rendered sample to the desired view.

4. Define two points. Hold **Crtl** and click to create the first point. **Crtl** + click to create a second point. Two white points appear on the image (fig.1).

5. Go to the **Measure Menu** > Select **Calculate Distance** to measure the distance between the two points.

6. The measured distance is displayed (fig.2).
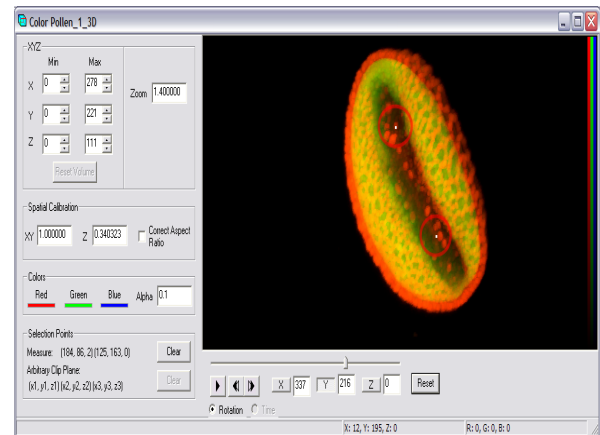
7. Click **Clear** to reset the two selected points.
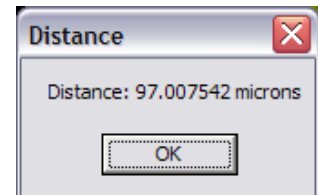
fig.1

fig.2

Calculating Visible Volume

1. Click and drag to rotate the rendered sample to the desired view.

2. Go to the **Measure** menu > Select **Calculate Volume** (fig.1).
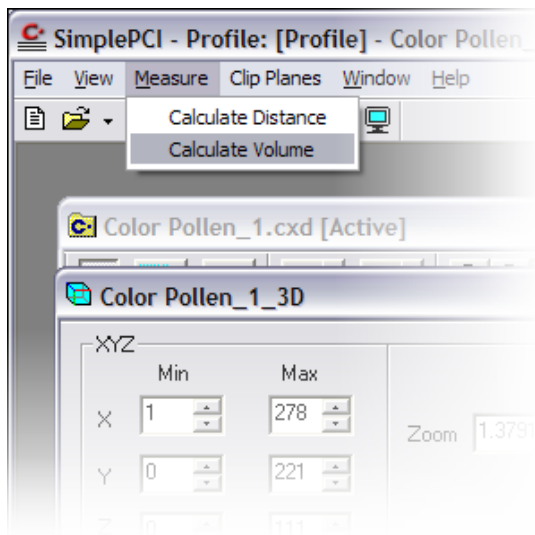
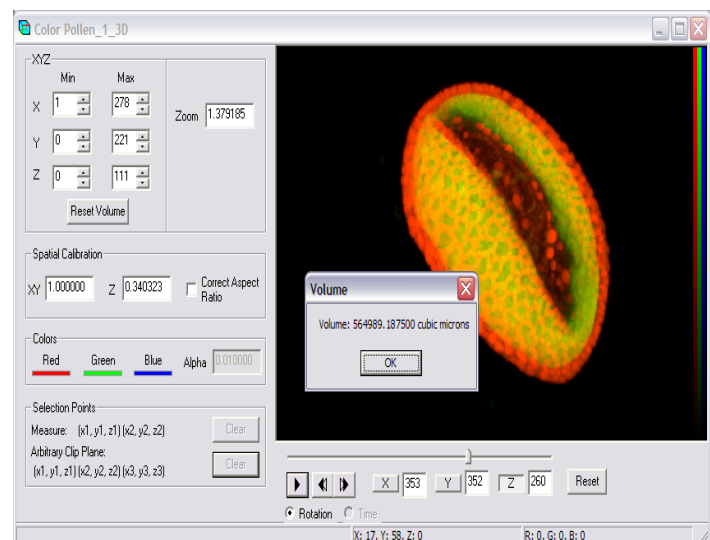3. The **Visible Volume** is displayed (fig.2).

fig.1

fig.2

Visualizing Colocalized Points

1. Open a data document. **File** > **Open** > **Data Document**> Select **File** > **Open**.

2. Click on the **Multi-dimensional Viewer** icon on the SimplePCI toolbar. The application starts transferring the Data into the 3D viewer.

3. Click and drag to rotate the rendered sample to the desired view.

4. Show colocalization. Go to the **View** > Select **Show Colocalized Points** (fig.1).

5. Colocalized points are displayed. Colocalized points are the colors emitted by the fluorescent molecules sharing the same pixel in the image (fig.2). Non-colocalized points are not displayed.
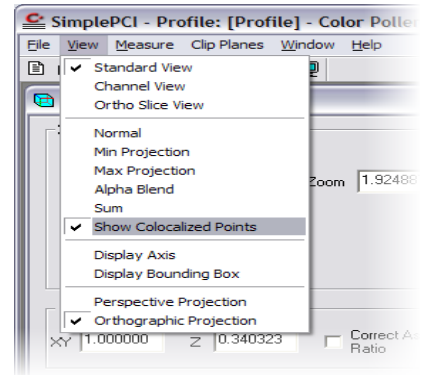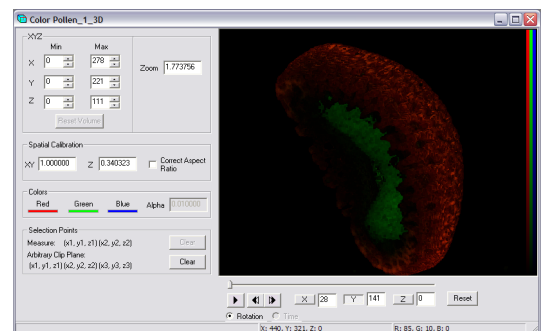

fig.1

fig.2

Importing 4D Data Set

1. Open the data document. File > **Open** > **Data Document** > Select **File** > **Open**.

2. Click on the **Group Selection** Icon located to the right of the playback toolbar.

3. Select **Multi-Dimensional Viewer Properties** from the **Multi-Dimensional Viewer** drop-down menu. (fig.1).

4. Click on **Group Mode** until you see the **All Groups** icon (fig.2).

5. Click on **Create MD View** to export the Z-series of each time-point.

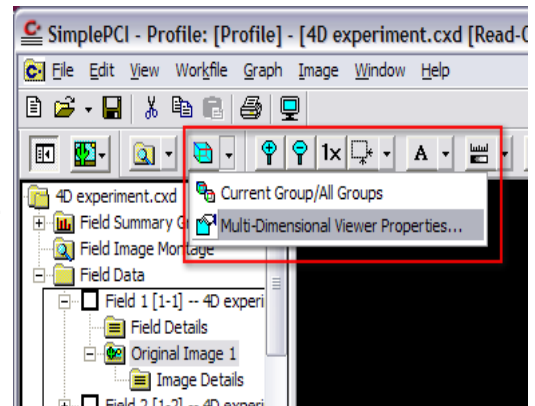6. Select **Time** in the 3D Viewer and drag the slider to view the Data over time (fig.3).
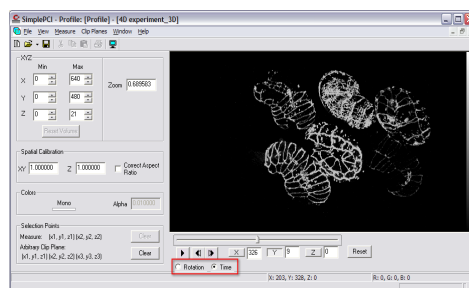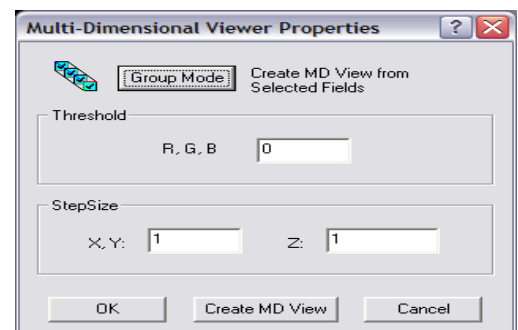

fig.1

fig.3

fig.2

Important Definitions

Channel View
Displays a single 2D projection of the 3D volume as seen from your perspective for each of the channels captured. Up to three channels are able to be displayed.

Ortho Slice View
This feature displays three single orthogonal planes, oriented to the object's XY, XZ, ZY perspectives. These planes may be adjusted by holding down the CTRL key, left-clicking on a plane, and dragging (while still holding the CTRL key).

Normal Projection
A single 2D projection of the 3D volume as seen from your perspective at the object's selected orientation.

Minimum Projection
This function takes parallel rays, perpendicular to the viewing surface, and casts them through the image. The minimum voxel value encountered along each ray is taken for the projection pixel value and the resulting image is made up of each minimum voxel value. The Minimum projection provides volumetric representations in which foreground intensities tend to be suppressed. This projection highlights edges and prominent dim features, and is typically used for viewing bright field images.

Maximum Projection
Find the maximum intensity along a projector. This projection highlights edges and prominent bright features, and is typically used for viewing fluorescence images.

Sum Projection
This function takes all the voxel values along each parallel ray, perpendicular to the viewing surface, and sums their intensity values. This creates a projection of all the summed values. Sum Projections provide volumetric representations of the dataset in which more information from the dataset is considered than with the Maximum projection. Background noise will also be included in this type of projection. It is useful for dark images

Alpha Blend
The Alpha channel specifies how a pixel's colors should be merged with another pixel when the two are overlaid, one on top of the other. The alpha channel contains a value ranging from 0 to 1. A value of 0 means that the pixel does not have any coverage information and is fully transparent. A value of 1 means that the pixel is fully opaque.

Colocalized Points
Display the colors emitted by fluorescent molecules sharing the same pixel in the image.

Display Axis
Display colored bars on the axes to make it easier to identify the orientation of the object. The X axis is red, the Y axis is green and the Z axis is blue.

Display Bounding Box
Outline the boundaries of the dataset around orthogonal planes, and around subvolume boundaries. This aids in judging the orientation of the dotted line and in judging the relative positions of the planes and the subvolumes.

Perspective Projection
A 2D representation of a 3D object (the data set) as it is perceived by the eye. The object is displayed in a depth perspective so that closer objects appear larger than distant ones.

Orthographic Projection
A 2D representation of a 3D object formed by the perpendicular intersection of lines drawn from points on the object to a plane of projection.