IN Carta

Image Analysis Software

3D Viewer User Guide

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MOLECULAR DEVICES

Product manufactured by Molecular Devices, LLC.
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Contents

Chapter 1: IN Carta Image Analysis Software

The IN Carta® Image Analysis Software provides powerful analytics for advanced phenotypic classification and 3D image analysis. It delivers robust, quantitative results from complex biological images and datasets using advanced AI technology.

Faster Data

- Intuitive design makes complex analysis accessible with minimal training.
- Shorten analysis time with true parallel processing.

Reliable Data

- Sophisticated algorithms generate reliable data with minimal user input.
- Improved segmentation algorithms represent cellular structures more accurately.

Results That Matter

- See real results quickly—from populations to single cells—using integrated data visualization tools.
- User-friendly interface guides you through your discoveries with continual updates that grow with your needs.

Terminology

The following table defines the terms and abbreviations used in this guide.

Term	Definition
3D Stack	Series of images taken in different Z planes of a specimen. Combines images to provide a composite image with a greater depth of field than the individual source images.
Mask	Image showing object segmentation
Voxel	A voxel represents a point within a 3D dataset similarly to how a pixel represents a point in a 2D image.
Z plane / Z slice	One of a set of 2D images (XY planes) that compose a 3D dataset, representing a single focal plane.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—www.moleculardevices.com/service-support—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Technical Support

To contact Molecular Devices Technical Support, submit a support request through the Molecular Devices Knowledge Base at support.moleculardevices.com.

You can also submit a support request by phone. For regional support contact information, go to www.moleculardevices.com/contact.

To expedite support, be prepared to provide the software version and your activation ID. To display this information, at the bottom left of the IN Carta window, click  **Settings**.

Documentation

Review the product documentation on the Molecular Devices Knowledge Base at support.moleculardevices.com. In addition, online Help is available within the IN Carta software.

Additional Resources

Web-based microscopy courses:

- www.microscopyu.com
- www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html

The *Molecular Probes Handbook* offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis:

- www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook.html

The *Assay Guidance Manual* details state-of-the-art approaches to high-content screening (HCS) and discusses challenges specific to HCS. It serves as a good introduction for new HCS practitioners.

- www.ncbi.nlm.nih.gov/books/NBK100913

About This Guide

This guide is intended for the scientist using the IN Carta software. It describes how to use the 3D Viewer Incorporated in the IN Carta software.

The information in this guide is valid for IN Carta software version 2.1 and is subject to change without notice. We recommend that you review the guide on the Molecular Devices Knowledge Base at support.moleculardevices.com for the most up-to-date information.

Chapter 2: 3D Viewer

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3D Viewer is available on the Image Viewer on the **Interactive: Analyze** page and **Interactive: Data** page. It provides the following functions:

- Enables visualization of single-channel and multi-channel 3D image stacks as a volume rendering.
- Enables mask generation with raw images.
- Enables inspection of 3D stacks, assessing segmentation accuracy and obtaining biologically relevant visualization of 3D samples.

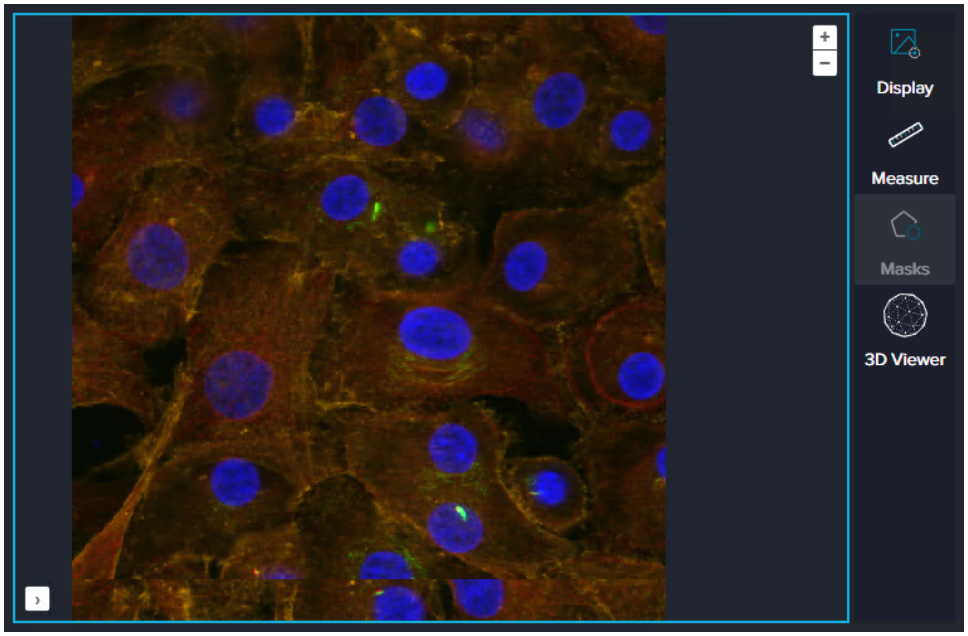
Note: An additional license is required to use 3D Viewer. The  **3D Viewer** control is not available without a valid license.

Interactive: Analyze (Volume Rendering Only)

To run the analysis with volume rendering only:

1. Create a new protocol or open an existing one. See the *IN Carta Help* for details.
2. On the **Interactive: Analyze** page, click  **3D Viewer**.

Note: An additional license is required to use 3D Viewer. The  **3D Viewer** control is not available without a valid license.



Interactive: Analyze (Volume Rendering with Optional Masks)

3D Viewer can generate mesh-like masks (3D masks) on demand using a separate algorithm after creating regular segmentation masks. If you select the **Save 3D masks** check box in the Custom Module Editor software, masks are generated automatically when you run the protocol. We recommend selecting the check box when segmentation settings are roughly optimized for a given 3D stack.

To run the analysis with additional masks:

1. Create a new protocol or open an existing one. See the Help for details.
2. Select one or more targets of interest and adjust analysis settings as needed.
3. Click **Apply** to create segmentation masks for selected targets.
4. (Optional) Click **Options**. On the **Z Stack** tab, click the **3D Options** drop-down and select the subset of Z slices in to analyze and load into 3D viewer.

   Note: By default, all Z slices from a current stack are rendered in 3D Viewer.

5. Click  **3D Viewer**.
6. If you did not select the **Save 3D masks** check box in the Custom Module Editor software, a dialog appears prompting you to create masks.
   Do one of the following:
   - Click **Yes** to generate masks and then open 3D Viewer.
   - Click **No** to open 3D Viewer without 3D masks.

Interactive: Data (Volume Rendering with Masks)

You can open 3D Viewer for datasets after analysis. If you selected the **Save 3D masks** check box in the Custom Module Editor software, the rendering includes masks. Otherwise, a dialog appears prompting you to create masks.

Do one of the following:

- Click **Yes** to generate masks and then open 3D Viewer.
- Click **No** to open 3D Viewer without 3D masks.

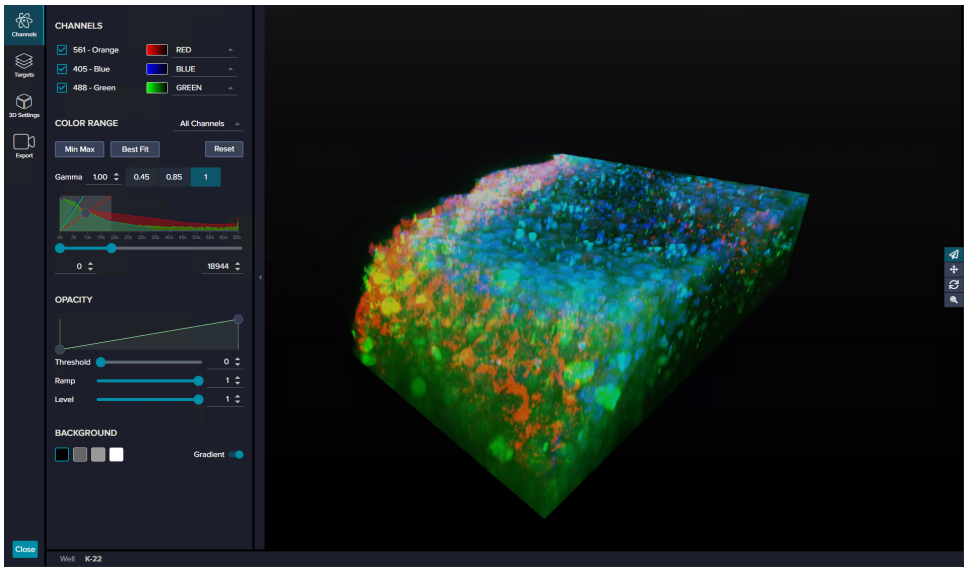
Note: If you specified a subset of Z slices in the protocol, then only those Z slices are rendered in 3D Viewer.

Chapter 3: 3D Viewer Window

When you start 3D Viewer, the selected stack is rendered. The rendering includes 3D masks, if available.

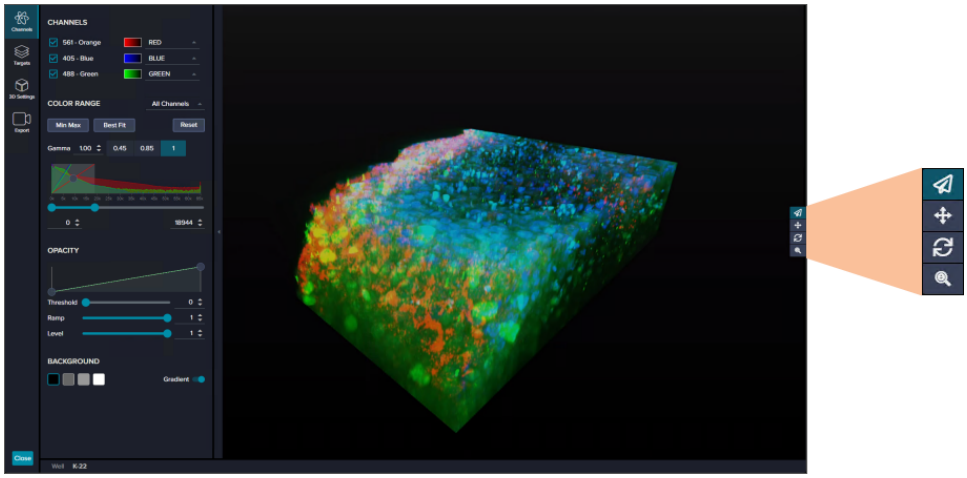
Note: When 3D Viewer is open, you cannot access the IN Carta software window.

Click **Close** to exit 3D Viewer and return to the IN Carta software window.



3D Viewer Navigation Controls

Use the navigation tools to rotate, pan, and zoom the volume and masks rendered in 3D Viewer.



The navigation tools include the following controls:

 **Examine**: Click to select the Examine tool, which combines Pan, Rotate, and Zoom into one tool. With the tool selected, use it to do the following:

- To rotate view: Hold the mouse button and drag the mouse.
- To pan: Hold **SHIFT** and drag the mouse.
- To zoom in: Scroll the mouse wheel up (or hold **CTRL** and drag the mouse to the right or up).
- To zoom out: Scroll the mouse wheel down (or hold **CTRL** and drag the mouse to the left or down)

The Examine tool is the universal tool. Use the other tools for more control to perform specific manipulations with the rendering.

 **Pan**: Click to select the Pan tool, which moves the rendering in 3D Viewer. With the tool selected, hold the mouse button and drag the mouse.

 **Rotate**: Click to select the Rotate tool, which rotates the rendering in 3D Viewer. With the tool selected, hold the mouse button and drag the mouse.

 **Zoom**: Click to select the Zoom tool, which zooms in and out of the rendering in 3D Viewer. With the tool selected, use it to do the following:

- To zoom in: Click the left mouse button and drag the mouse to the right or up.
- To zoom out: Click the left mouse button and drag the mouse to the left or down.

3D Viewer Tabs

The 3D Viewer window includes the following tabs:

Channels: See Channels Tab on this page for details.

Targets: See Targets Tab on page 16 for details.

3D Settings: See 3D Settings Tab on page 17 for details.

Export: See Export Tab on page 19 for details.

Channels Tab

Use the **Channels** tab controls to adjust the display of raw image data (volume). It includes the following sections:

- Channels, see below
- Color Range, see page 12
- Opacity, see page 14
- Background, see page 15

Note: You can set the contrast or highlight of lower intensity values for either a selected channel or all channels simultaneously.

Channels

In the **Channels** section, select the channels displayed in 3D Viewer. Unselected channels are hidden.

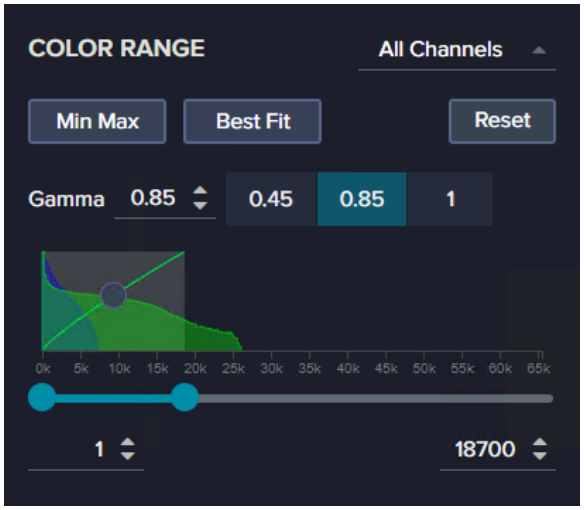
Channel colors are set based on the color selections made in the main IN Carta window.

Click the drop-down to modify the color for each channel in 3D viewer, if needed. In the color drop-down, select **Custom** to define a new color.

Color Range

In the **Color Range** section, adjust the contrast displayed in 3D viewer.

By default, color range adjustments apply to **All Channels**. To set the display settings for an individual channel, select a channel from the drop-down.



The **Color Range** section includes the following controls:

- **Min Max**: Click to set the **Min** and **Max** values to the minimum and maximum voxel intensities of an active channel.
- **Best Fit**: Click to set optimal display values based on the histogram of an active channel.
- **Reset**: Click to restore all Color Range values to their default settings.
- **Gamma**: Set a value to highlight or hide low intensity voxels. Values less than 1 highlight dim voxels; values greater than 1 cause bright voxels to appear even brighter and hide dim voxels.

Tip: You can also adjust the Gamma value by clicking and dragging the handle in the Color Range graph. Note that the cursor changes to a vertical two-headed arrow.

- **Min**: In the bottom left field, enter the minimum Color Range value. All voxels with intensities below the **Min** value display in black (and are hidden). Increase the **Min** value above the background or any other dim structures.
- **Max**: In the bottom right field, enter the maximum Color Range value. All voxels with intensities above the **Max** value display in white (or the selected color). Increase the **Max** value so that the rendering is bright enough to view all the biological structures of interest.

Tip:

- You can also adjust the **Min** and **Max** values by clicking and dragging the shaded area in the Color Range graph. Note that the cursor changes to a horizontal two-headed arrow.

- Another way to adjust the **Min** and **Max** values is to click and drag the slider handles below the Color Range graph.

Opacity

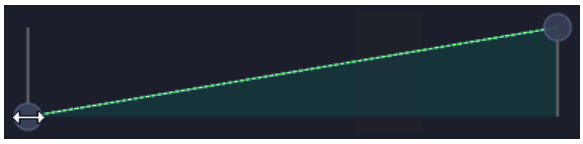
In the **Opacity** section, adjust the settings to achieve optimal display across the channels.

By default, color range adjustments apply to **All Channels**. To set the display settings for an individual channel, select a channel from the drop-down in the Color Section above. See Color Range on page 12 for details.

The **Opacity** section includes the following controls:

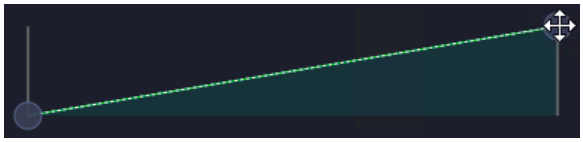
- **Threshold**: Set a value or drag the slider to control which voxels are hidden based on their intensity values. Increasing the value hides more low intensity signal (similar to the **Min** in the Color Range section).

  Tip: You can also adjust the **Threshold** value by clicking and dragging the left handle in the Opacity graph. Note that the cursor changes to a horizontal two-headed arrow.

- **Ramp**: Set a value or drag the slider to control the contrast of a rendering. Decreasing the value increases the contrast.

  Tip: You can also adjust the **Ramp** value by clicking and dragging the right handle in the Opacity graph to the left and right. (Dragging up and down adjusts the **Level** value.) Note that the cursor changes to a four-headed arrow.

- **Level**: Set a value or drag the slider to control the brightness of the displayed rendering. Decreasing the value decreases the brightness.

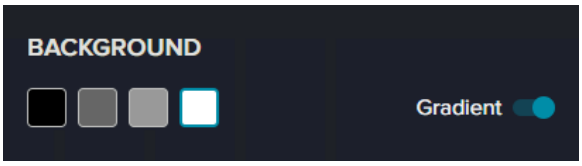
Tip: You can also adjust the **Level** value by clicking and dragging the right handle in the Opacity graph up and down. (Dragging to the left and right adjusts the **Ramp** value.) Note that the cursor changes to a four-headed arrow.

Background

In the **Background** section, adjust the background color in 3D Viewer.

Note: The background color setting is the same on the Channels tab and Targets tab. When a setting is changed on one tab, that change is reflected in the other tab.

By default, the background color is black, and the **Gradient** is set to on (to include shading).



Tip:

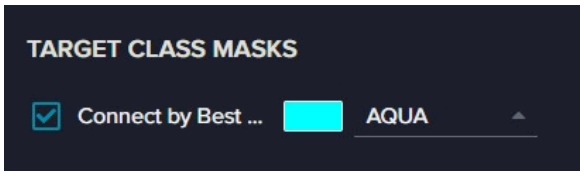
- Set **Gradient** to off to achieve better contrast.
- Set the background color to white to improve visualization of transmitted imaging modalities (for example, phase contrast or DIC).

Targets Tab

Use the **Targets** tab to set options for visibility of target masks generated during analysis.

Target Class Masks

In the **Target Class Masks** section, you can control the visibility and color assignments for target masks.

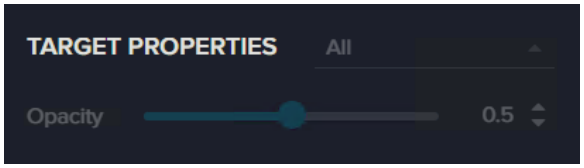


Click the drop-down to modify the color for each channel in 3D viewer, if needed. In the color drop-down, select **Custom** to define a new color. You can also randomize color assignment across all objects for a given target mask. This is helpful to evaluate splitting of adjacent objects.

Target Properties

In the **Target Properties** section, adjust the transparency of target masks.

By default, target property adjustments apply to **All** target masks. To set the display settings for an individual target masks, select a target from the drop-down.



The **Target Properties** section includes the following controls:

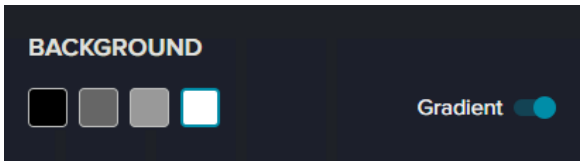
Opacity: Set a value for the transparency of target masks by moving the slider or entering a value from 0 to 1.

Background

In the **Background** section, adjust the background color in 3D Viewer.

Note: The background color setting is the same on the Channels tab and Targets tab. When a setting is changed on one tab, that change is reflected in the other tab.

By default, the background color is black, and the **Gradient** is set to on (to include shading).



Tip:

- Set **Gradient** to off to achieve better contrast.
- Set the background color to white to improve visualization of transmitted imaging modalities (for example, phase contrast or DIC).

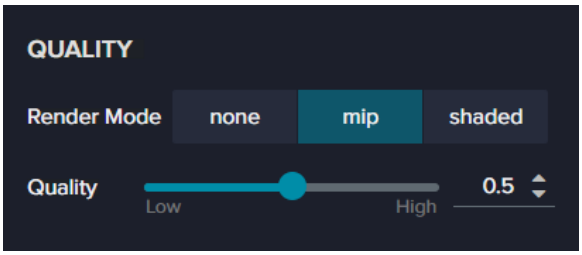
3D Settings Tab

Use the **3D Settings** tab to adjust general display settings in 3D Viewer and display cross-sectional view of the rendered image. It includes the following sections:

- Quality, see below
- Clipping, see page 18
- Camera Presets, see page 18

Quality

In the **Quality** section, set options for the render mode of channels and the display quality.



The **Quality** section includes the following controls:

- **Render Mode**: Click to select one of the following modes to render the image:
  - **none**: Hides channels (volume) and displays only target masks. Use this option as a quick way to visualize masks without individually deselecting the channels tabs.
  - **mip**: Renders a maximum intensity projection. This option is optimal for most visualizations.
  - **shaded**: True volumetric rendering with shading, however it can show excessive levels of details.
- **Quality**: Set the level of detail in the rendering. Higher settings require an advanced graphics processing unit (GPU) and can result in lag while viewing the rendering.

Clipping

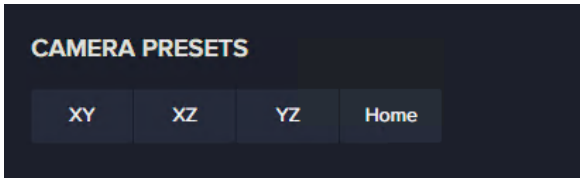
In the **Clipping** section, adjust the plane to generate an interactive cross-sectional view of channels and/or target masks. Set the toggle to on (shown in the on position) to make the controls available.

The **Quality** section includes the following controls:

- **Presets**: Click to select the clipping orientation, either **XY**, **XZ**, or **YZ**.
- **Position**: Set a value to move across the rendering.
- **Angle 1** and **Angle 2**: Set values to control tilt.
- **Clip:** Click to select an option, either **Targets** (target masks only), **Volume** (channels only) or **Both** (channels and target masks).
- **Opacity**: Set a value to adjust for optimal opacity.
- **Color**: Click to select the color of the clipping plane.

Camera Presets

In the **Camera Presets** section, select the pre-defined rotation position of the 3D rendering.



Select one of the following options:

- **XY**: Equivalent to view from the top.
- **XZ**: Orthogonal (sideways) view along XZ plane.
- **YZ**: Orthogonal (sideways) view along YZ plane.
- **Home**: Initial view of the rendering when you open 3D Viewer.

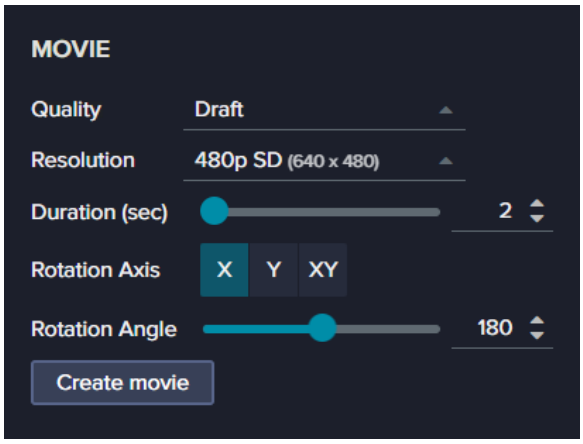
Export Tab

Use the **Export** tab to export still and animated renderings of the 3D Viewer display. It includes the following sections:

- Movie, see below
- Screenshot, see page 20

Movie

In the **Movie** section, you can export an animated (rotated) visualization of single- and multi-channel rendering from 3D Viewer as an MP4 file with or without 3D target masks.



The **Movie** section includes the following controls:

Quality: Click to select the quality of the movie. Better quality provides greater detail, but requires more time to generate. The following options are available:

- **Draft**
- **Low**
- **Medium**
- **High**
- **Ultra**

Resolution: Click to select the resolution for the movie. The following options are available:

- **480p SD (640 × 480)**
- **720p HD (1280 × 720)**
- **1080p Full HD (1920 × 1080)**
- **1440p QHD (2560 × 1440)**

Duration (sec): Set the length of the movie between 2 and 60 seconds (that is, the amount of time in the movie for the image to rotate to the specified angle. In a longer movie, the image rotates more slowly, allowing you to observe greater detail. However, a longer movie requires more time to generate and has a larger file size.

Rotation Axis : Click to set the axis around which the image will rotate. Select the axis that achieves the desired perspective during the rotation. The following options are available:

- **X**
- **Y**
- **XY**

Rotation Angle: Set the distance the rendering rotates (in degrees) during the specified duration. The available range is from 0° to 360°.

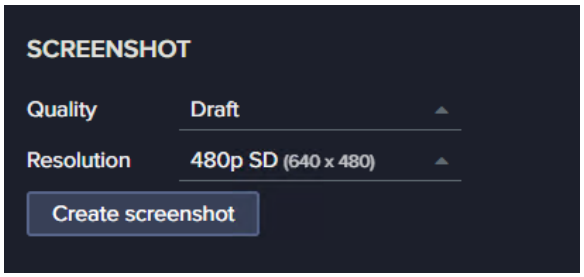
Exporting a Movie

To export a movie:

1. Set the controls on the **Movie** tab as needed.
2. Click **Create movie**.
3. When movie creation is complete, click **Download** to save it to the Downloads folder.

Screenshot

In the **Screenshot** section, you can export a still image from 3D Viewer as a TIFF file.



The **Screenshot** section includes the following controls:

Quality: Click to select the quality of the screenshot. Better quality provides greater detail, but requires more time to generate. The following options are available:

- **Draft**
- **Low**
- **Medium**
- **High**
- **Ultra**

Resolution: Click to select the resolution for the screenshot. The following options are available:

- **480p SD (640 × 480)**
- **720p HD (1280 × 720)**
- **1080p Full HD (1920 × 1080)**
- **1440p QHD (2560 × 1440)**

Exporting a Screenshot

To export a screenshot:

1. Set the controls on the **Screenshot** tab as needed.
2. Click **Create screenshot**.
3. When screenshot creation is complete, click **Download** to save it to the Downloads folder.

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