Octopus Software User Guide

Version 1.3.0

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Table of Contents

Introduction

The Octopus software has been developed to operate in conjunction with either Resolution Optics Submersible or Desktop holographic microscopes. The Resolution Optics Submersible microscope is a holographic microscope which can operate while fully submerged in water. The Desktop microscope is a portable benchtop instrument.

This user guide is strictly related to the installation, use and functionality of the Octopus software. For a detailed description of the setup and operation of either the Submersible or Desktop microscopes, please refer to the respective user guides.

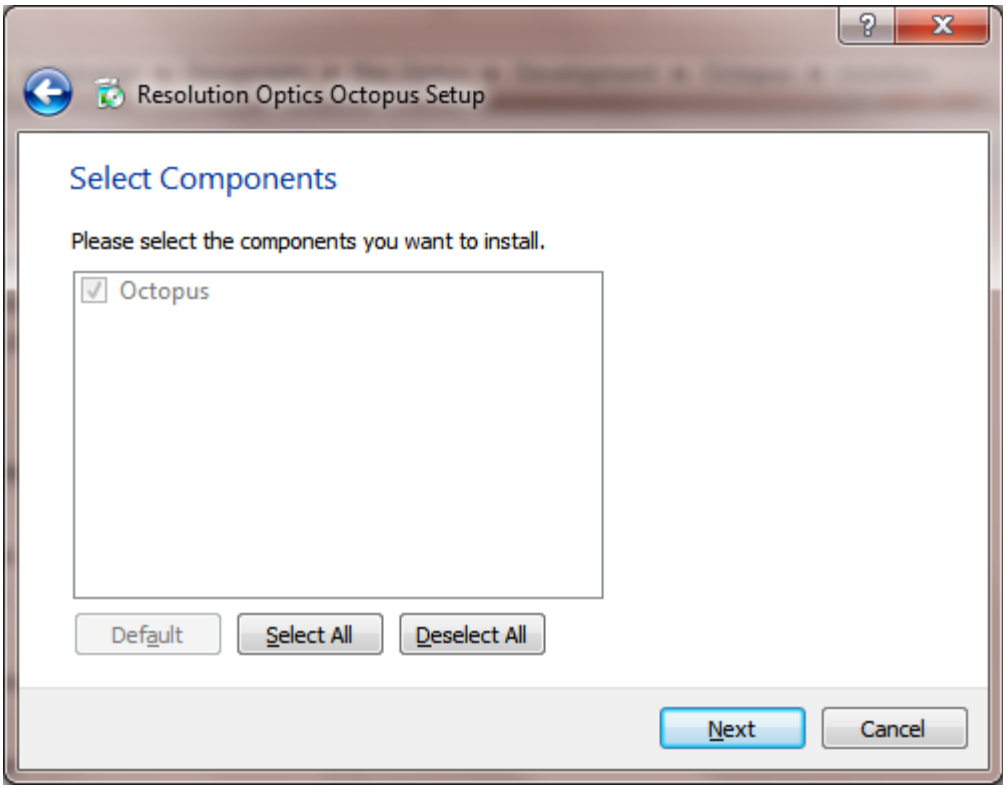
The Octopus software can be used for example in

- Marine research: water profiling, algae, plankton, phytoplankton
- Biological research: cell biology, neuroscience, capturing dynamic motion, 3D and 4D
- Water quality and monitoring: microorganism imaging
- Algae production: algae profiling

Quick Installation Guide

To install Octopus software on your computer

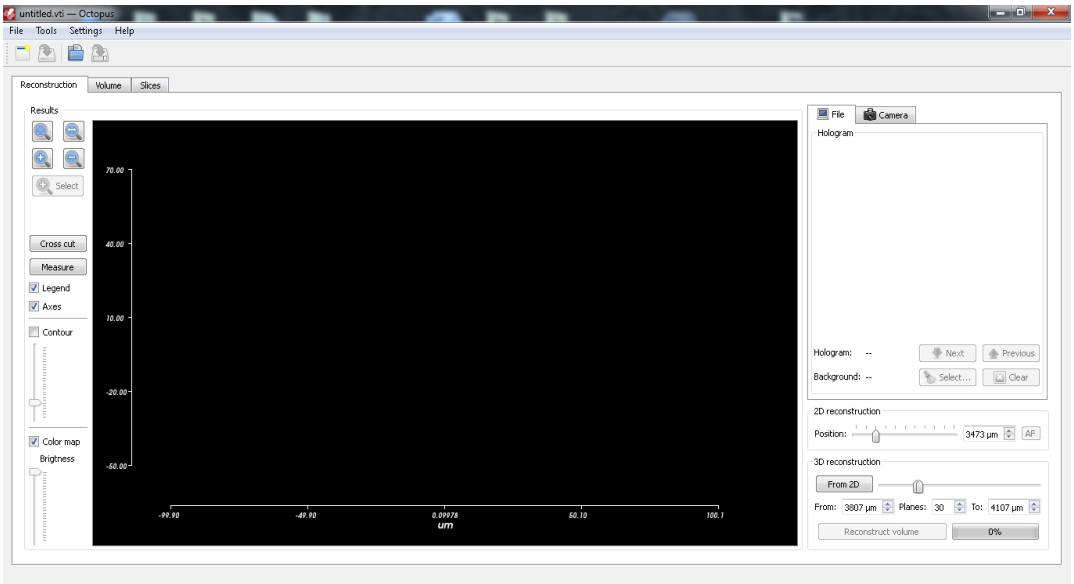
1. Insert the installation CD, that was provided, in your computer.
2. Run HaspUserSetup.exe. Follow the onscreen instructions.
3. Run the ebus_sdk_xxx.exe, follow the onscreen instructions.
4. Install Octopus by running OctopusInstaller.exe and following the onscreen instructions. Selecting the default parameters should typically be acceptable for most installations.



Octopus installer, selection of the installation package

Connection of the HASP dongle and starting Octopus

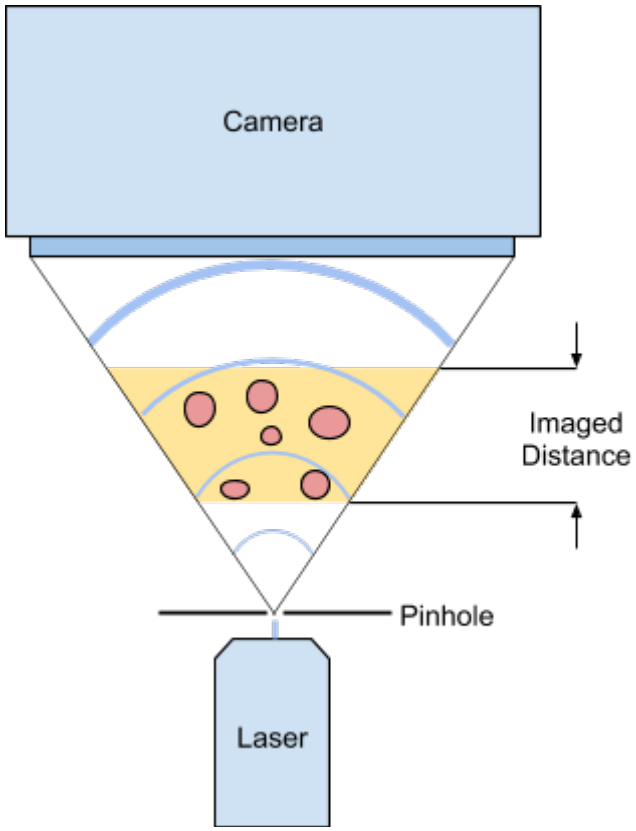
After installation, attach the supplied HASP hardware protection key (dongle) to a computer USB port. Make sure the dongle light turns on. Launch Octopus by going into `Windows Start Menu->Octopus`. The Octopus software will start.



Octopus Software user interface after launch

Principle of Operation

The Octopus software works with the Submersible or Desktop microscopes, which operate on the principles of holography to image a volume in magnification. A 405 nm laser is focused on a 500 nm pinhole of the same order of magnitude as the wavelength of the light, which produces a spatially coherent light source as a reference wave. Light which scatters from the objects within the media (water) will interfere with the reference wave to produce an interference pattern which contains spatial and phase information of the objects within the volume. This interference pattern, the hologram, is recorded by a CCD camera, and reconstructed mathematically to build images of the objects within the volume.



The basic principle of digital in-line holographic imaging

The reconstructed images can be further analysed, saved, or assembled into the volume reconstructions based on the application.

User Interface

Main Menu

File

Reconstruct Holograms - select the hologram file for reconstruction. PNG, JPEG, TIFF and BMP image formats are supported. You can either select (i) a single hologram to be reconstructed, or (ii) 2 images that will be used as a hologram/background pair during the reconstruction, or (iii) 4 or more images to reconstruct a hologram series. When hologram series is being reconstructed, multiple holograms are combined according to the rule (H2-H1) + (H3-H2)... Hologram series reconstruction is useful for 4D reconstructions to estimate particle velocities and microorganism swimming patterns.

Save Reconstructed Plane - saves reconstructed hologram image to the image file. PNG and BMP formats are supported.

Open Volume - opens previously saved reconstructed volume file. VTK volume files and BIORAD PIC files are supported.

Save Volume - saves reconstructed volume to file. VTK volume files are supported.

Start Video Recording – the results of the reconstruction will be saved to the selected movie file (AVI or MPG file format).

Stop Video Recording – ends video recording session, closes video file.

Print – prints the current view of the reconstruction.

Quit - closes the Octopus software.

Tools

2D Object Detection - opens the 2D Object Detection dialog. Refer to the dialog description for details.

3D Object Detection - opens the 3D Object Detection dialog. Refer to the dialog description for details.

Settings

Camera Options - opens the Camera Options dialog. Refer to the dialog description for details.

Edit Color map – opens the edit color map window.

Reconstruction Options - opens the Reconstruction Options dialog. Refer to the dialog description for details.

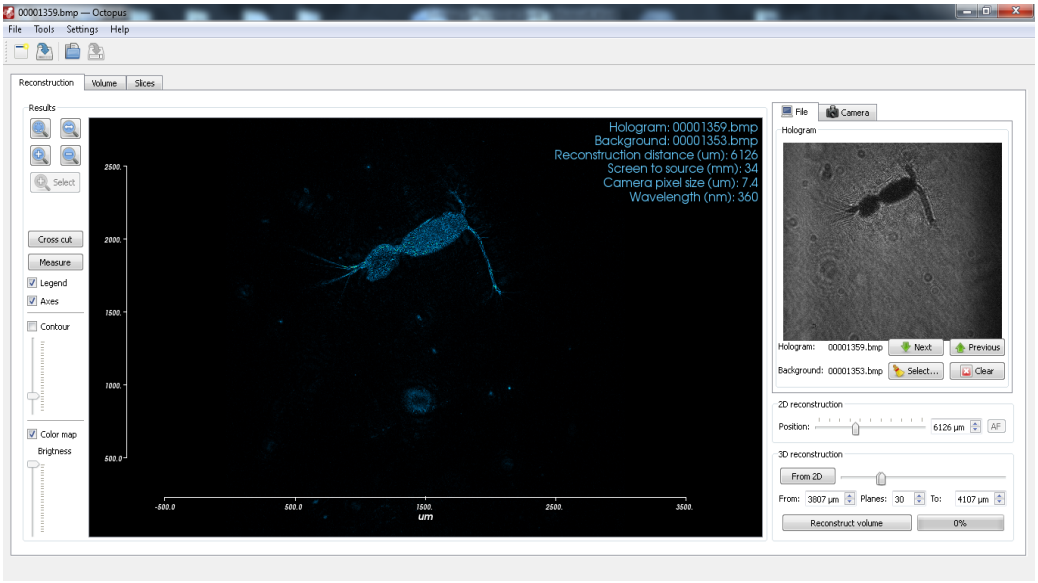
Help

About - shows information about the software.

Main Window

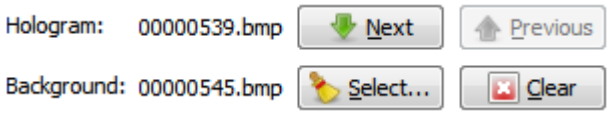
Reconstruction Tab

The reconstruction tab provides a means to preview the loaded hologram, select the background hologram for subtraction, reconstructing holograms in 2D, and reconstructing 3D volumes.



Reconstruction tab

To control what Hologram is being reconstructed, use the `Next` and `Previous` buttons. These buttons select the next / previous hologram for reconstruction in the current folder.



Background `Select` button allows to select the background hologram that will be subtracted from the current hologram prior to reconstruction. Subtracting backgrounds is highly recommended to eliminate noise and artifacts from the reconstructed images. The background image should be the same size as hologram image for subtraction to work.

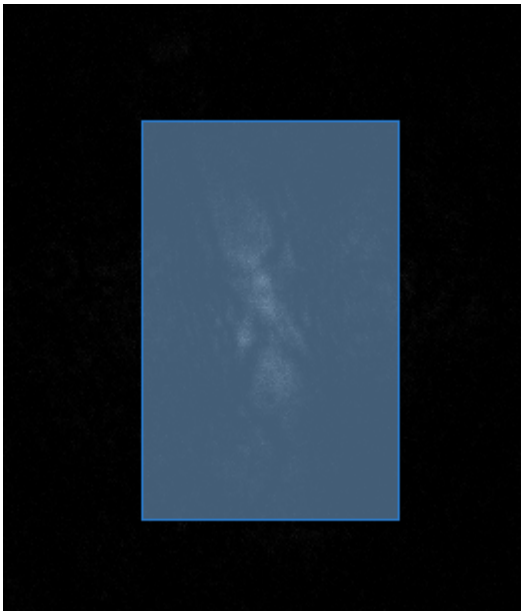
`Clear` button removes the background hologram and reconstructs the hologram with no background subtraction.

2D Reconstruction Panel

This panel controls the parameters of 2D reconstruction. The reconstruction position (in µm from the point source) along the z-Axis can be controlled with either the position slider, or the position input field. Change the position and hologram will be automatically reconstructed at this new position.

`AF` button controls the "Autofocus" feature. Autofocus allows you to quickly determine the position within the volume at which the selected object is in the optimal focus. To initiate the autofocus:

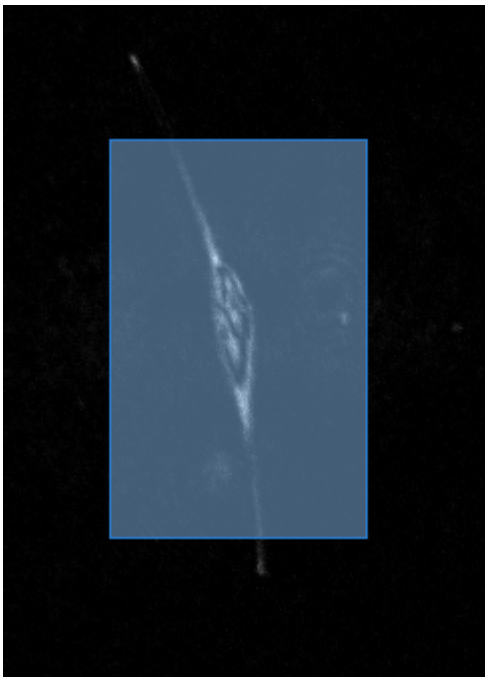
1) Select the object of interest in the Result View by left-clicking and dragging with mouse. Note the autofocus will search for the optimal focus from the currently selected reconstruction position.



Object out of focus

2) Press the `AF` button, or press `Ctrl-A` on keyboard.

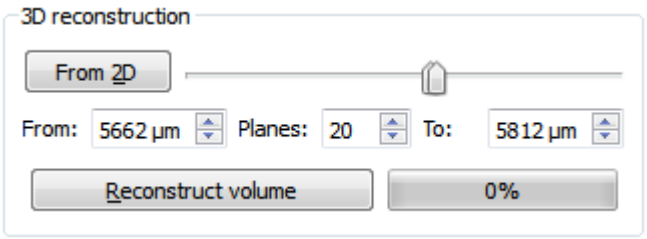
3) The object will come into the optimal focus. In certain cases, pressing the AF button for a second time improves the results.



Object from above in focus

3D Reconstruction Panel

3D Reconstruction Panel controls the parameters for the 3D (Volume) reconstruction.



`From` and `To` input fields, and linked sliders allow you to select the z-Axis boundaries of the volume (in µm from the point source).

`Planes` input field determines the number of reconstructions that will be performed to build the volume. The reconstructions are done at fixed intervals: (To - From) / (Number of Planes - 1).

The `From 2D` button copies the current position from the 2D Reconstruction Panel to the `From` value in the 3D Reconstruction Panel.

This allows for the quick transfer of positions between 2D and 3D reconstructions.

The `Reconstruct volume` button will start the volume reconstruction. The reconstruction mode, and volume reconstruction options can be set in the `Reconstruction Options` dialog. After the volume reconstruction, the software will automatically select the Volume Tab and show the reconstructed volume.

Control of the Results View zoom

The Results view zoom can be controlled with the respective buttons:

 Sets the reconstruction to the Fit to window mode. The whole content of the reconstructed hologram image will be shown.

 Sets the reconstruction to the Full scale mode. The reconstructed hologram image will be scaled such that 1 pixel of the image corresponds to the 1 pixel on the screen.

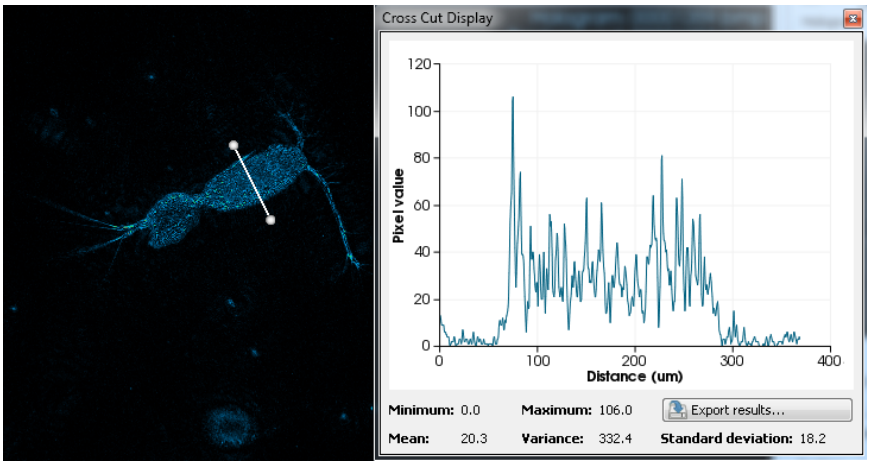
 Zooms the results view in.

 Zooms the results view out.

Zooms to selected region.

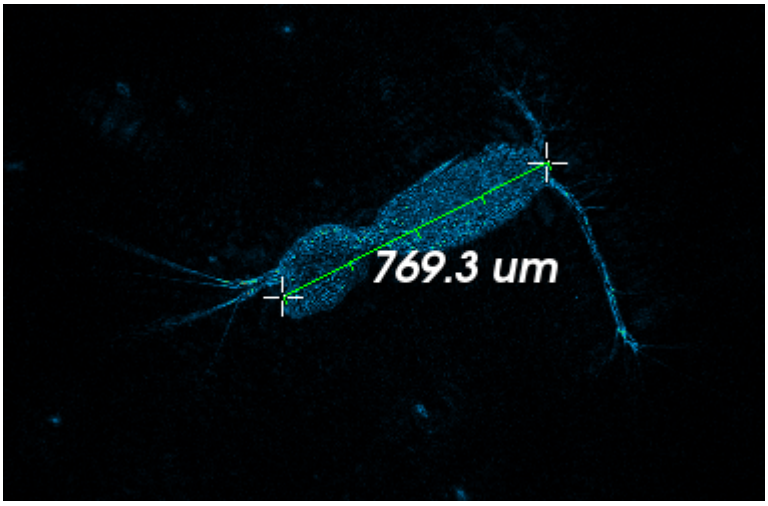
Cross Cut

The `Cross cut` button produces a line on the reconstructed image and a graph of the intensity values along the line. Click and drag the bollards to move the line. Statistics are presented, and the cut data can be exported to MS Excel file.



Measure

With the `Measure` button, clicking on the reconstructed image will start a measurement of the image, clicking again will set the measurement. The ruler can be moved by clicking on the end cross and dragging.

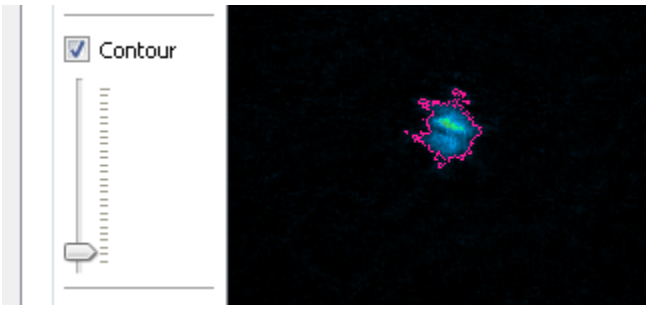


Legend & Axes

Checking the boxes will produce the legend on the upper right of the reconstructed image, and the axes along the lower and left hand side of the image. Axes and legend are saved on the images.

Contour

Checking the contour box will produce intensity based contours, which can be controlled using the slider.

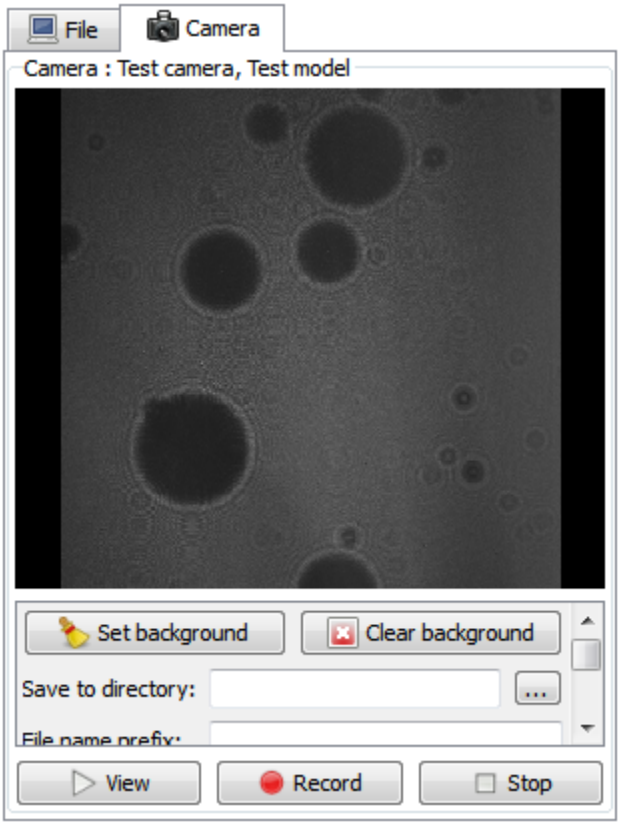


Color Map

A false color map based on intensity values is produced when this is checked. Control over the color map is found in the Settings menu.

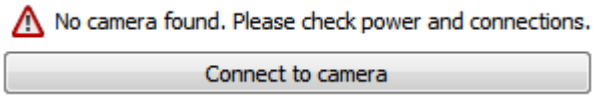
Camera

In addition to the reconstruction of the hologram image files, Octopus can reconstruct live images that are coming from the connected camera in real time. The cameras installed in Resolution Optics Submersible and Desktop microscopes are supported out of the box. For support of the 3d party cameras in custom holographic setups contact a Resolution Optics representative or support line.



Camera view and controls

Clicking on the camera tab will automatically activate the camera. If camera is not connected, check that the camera cables are properly attached, and power is on. Click `Connect to Camera` button to retry the connection.



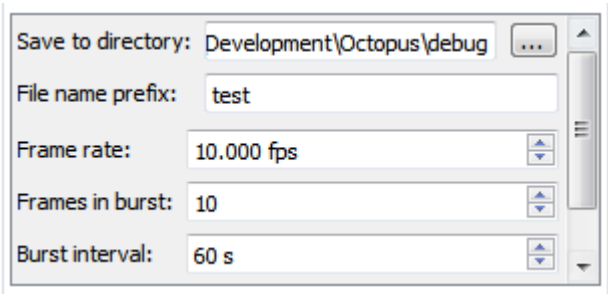
The live view from the camera will be appearing in the preview and hologram reconstructions will be performed in real time at the rate of up to 16 frames per second.[1] Higher frame rates are possible with reduced image sizes. All reconstruction parameters can be applied to the real time reconstructions, including the reconstruction position and mode.

Camera background frame control:

[1] Depending on CPU and GPU speed of the host computer.

Set background - uses current frame acquired by the camera as a background frame for reconstructions. The background frame will be subtracted from all the subsequent frames acquired by the camera.
Clear background - clears the background frame. All subsequent frames will be reconstructed without background subtraction.

Camera parameters:



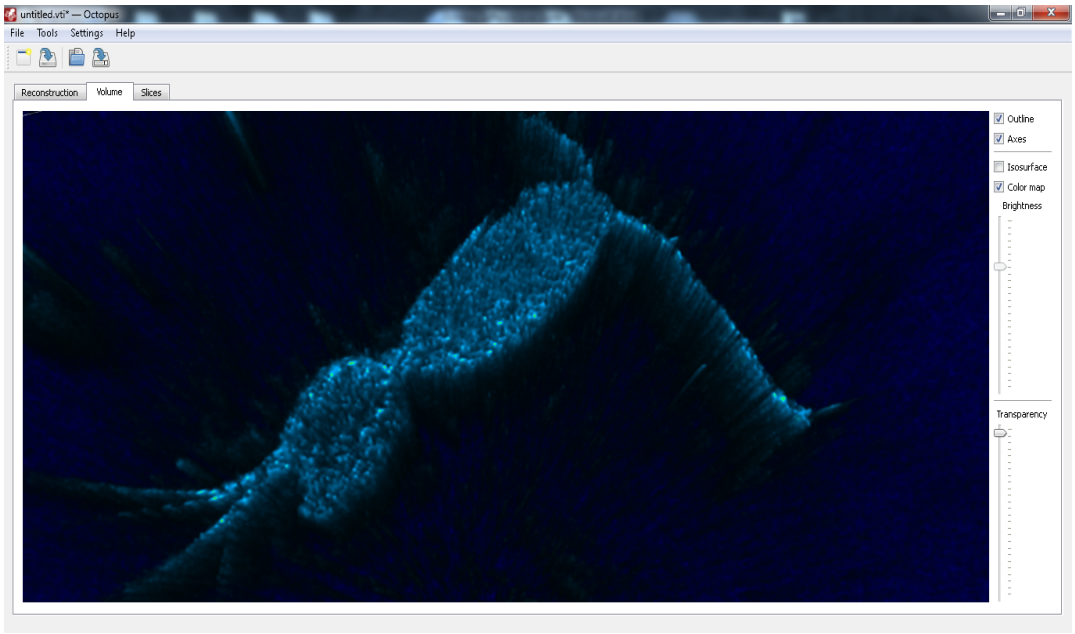
Save to directory - select the directory to which the images will be saved (in BMP format).
File name prefix - each image file captured by camera will be assigned a name prefix.
Frame rate - the rate of image capture in frames per second.
Frames in burst - number of frames saved in a single burst. If 0, frames will be saved continuously at frame rate. Note that burst only affects the saving of image files, the live view and reconstructions are not affected by the burst settings.
Burst interval - interval between bursts (in seconds).
Gain/Auto Gain - manual or automatic gain control of the camera.
Exposure/Auto Exposure - manual or automatic exposure control of the camera.

Camera controls:

View - activates live feed from the camera, starts reconstructions. The images captured by the camera will not be saved.
Record - activates live feed from the camera, starts reconstructions, saves captured images.
Stop - stops camera feed, reconstructions and image saving.

Volume Tab

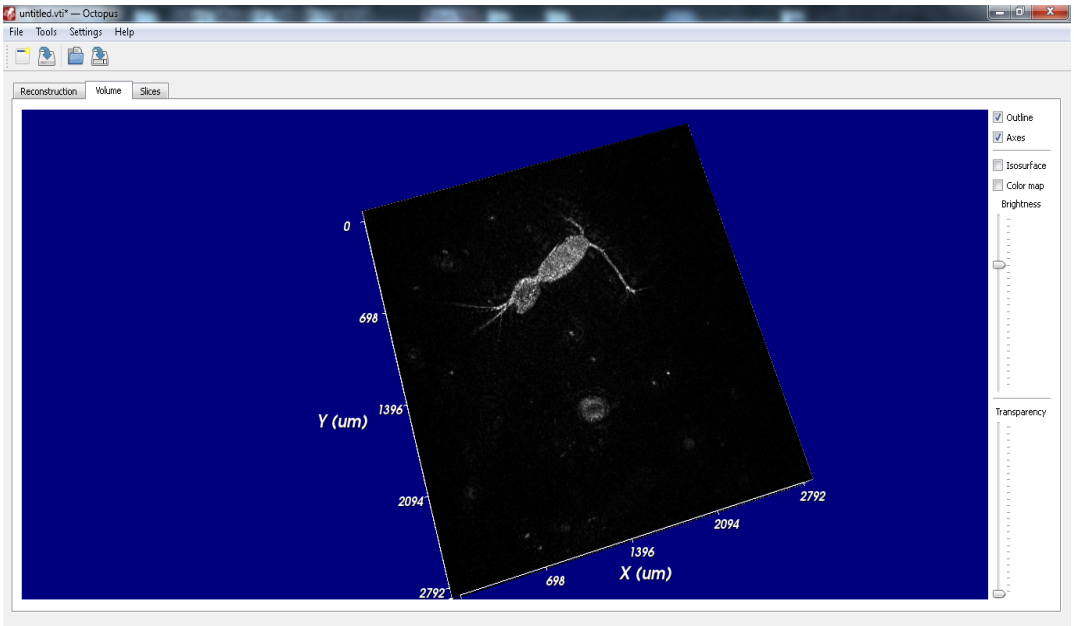
After 3D reconstruction, the reconstructed volume is shown in the Volume Tab.



3D navigation options:
Rotate - Press left mouse button and rotate the 3D scene by moving the mouse.
Zoom - Press right mouse button and drag up and down to zoom in and out. You can also use mouse wheel for zooming.
Pan - Press Shift, left mouse button and pan the scene by moving the mouse.
Reset zoom and pan - Press the "R" key.

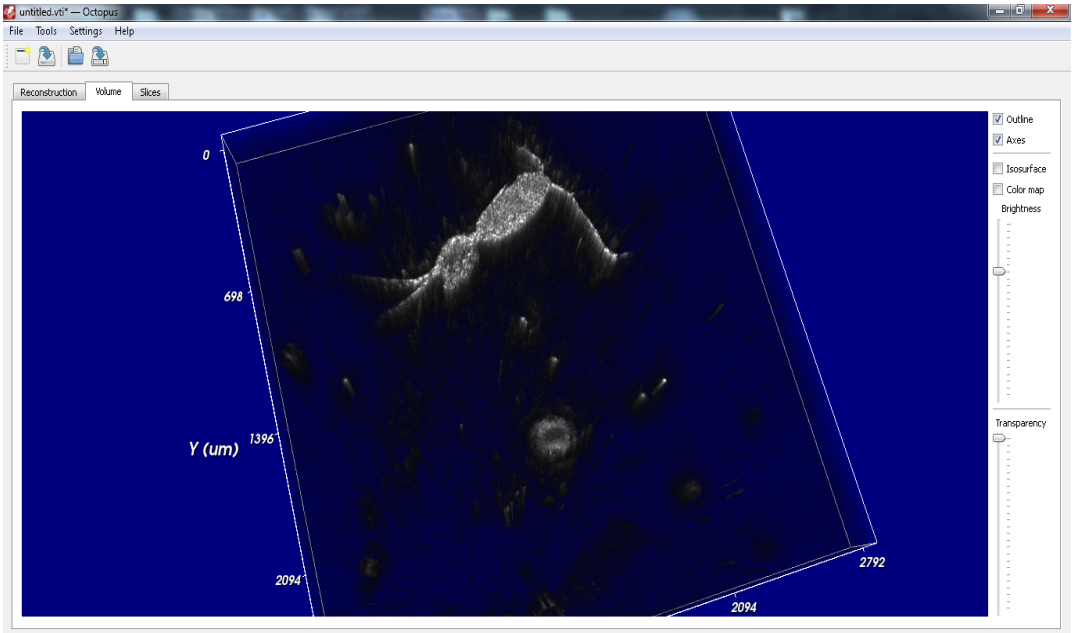
3D view options:
By default, the Volume view is shown with 0 opacity, in grayscale mode.

The brightness of the objects can be controlled by the brightness slider.



Volume View, grayscale mode, 0% transparency

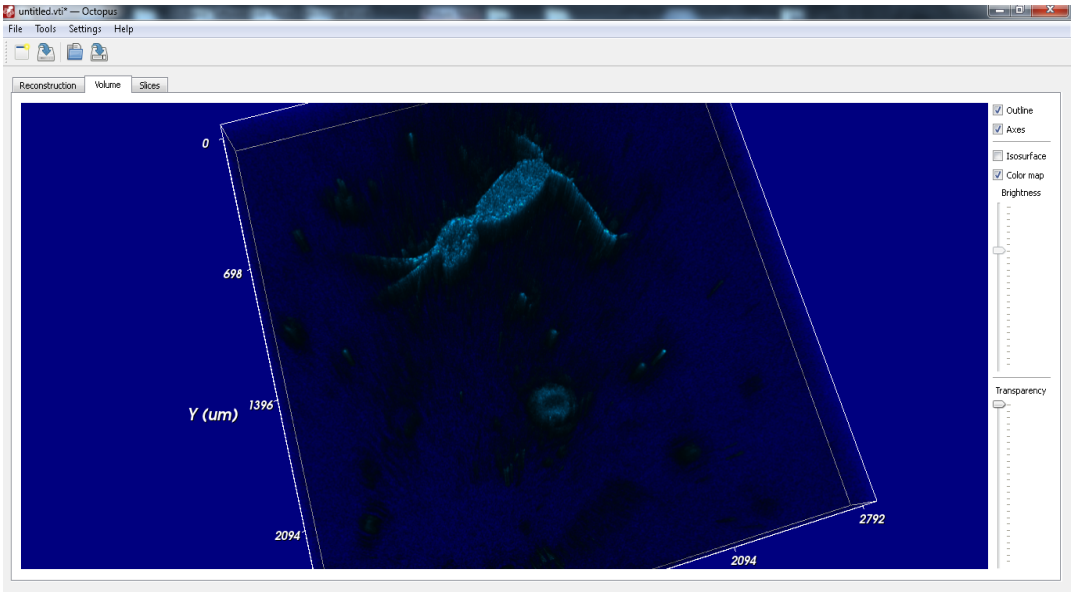
To change the transparency level, adjust the transparency slider. Black voxels in the volume will become transparent. The degree of transparency is controlled by the slider from 0 to 100%.



Volume View, grayscale mode, 100% transparency

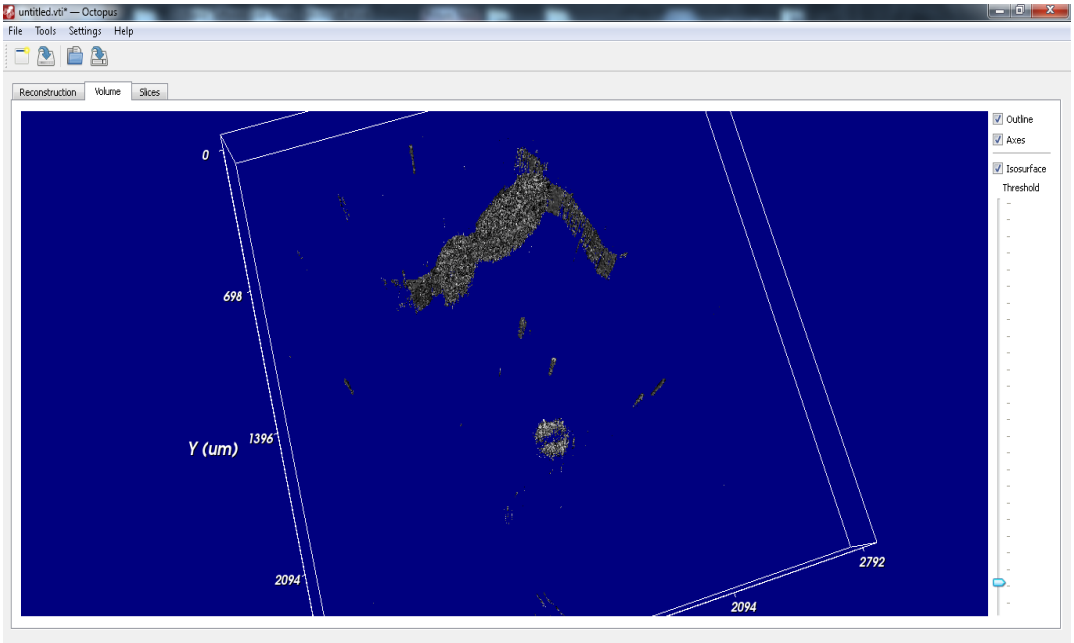
To activate the Color map feature, select the Color map check box. The artificial color map will be applied to the volume. You can

simultaneously control the degree of transparency with the transparency slider.



Volume View, colormap mode, 100% transparency

To activate the isosurface view, select the Isosurface checkbox. The isosurface produces the solid surface from the connected voxels with the same value. The value threshold can be adjusted with the slider.

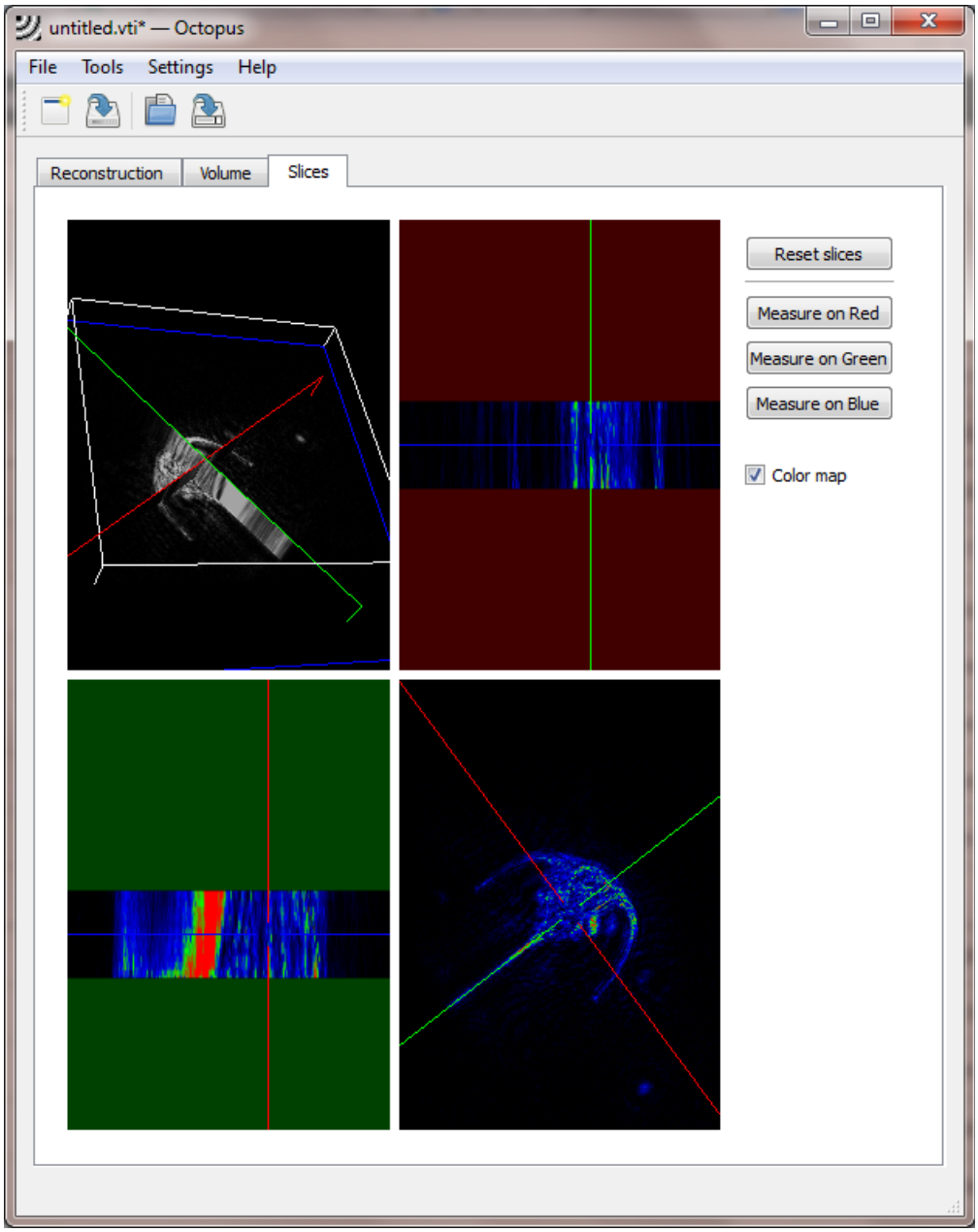


Volume View, isosurface mode

All the options selected in the Volume view will be saved upon Octopus exit and recalled on the next launch.

Slices Tab

The slices allow you to slice through volume using the oblique planes. The 3 panels with red, green and blue background correspond to the red, green, and blue slice planes. The top left panel shows the slice planes in the context of the overall volume.

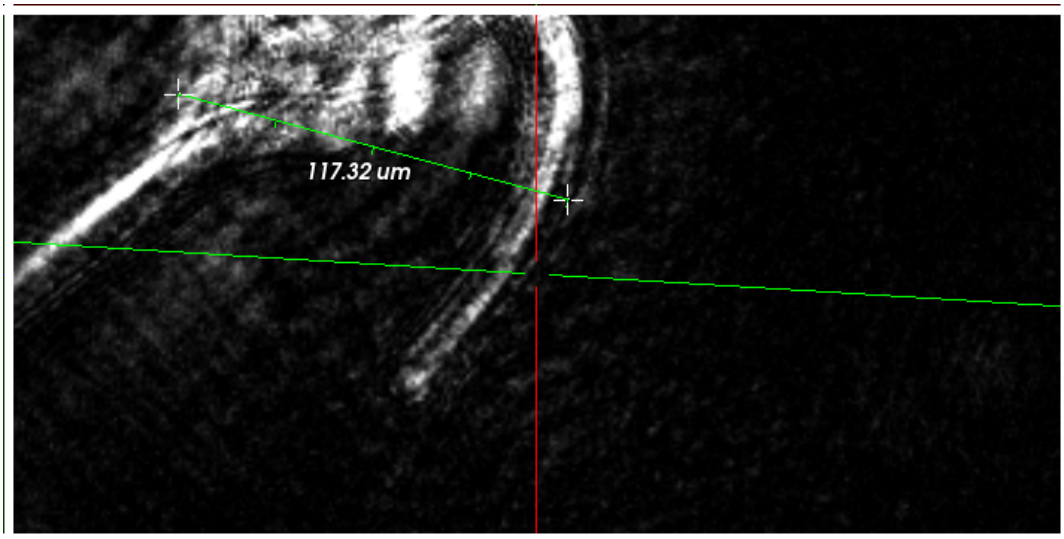


To adjust the slice plane position, select the point where the 2 planes cross (the cursor will change shape and become a cross cursor) and drag the planes around.

To adjust the angle between the planes, grab one of the planes (the cursor will become a hand cursor) and drag it with the mouse.

As you adjust the plane position and angle, the view will change in the respective panel(s) to show the cross-cut of the volume with the plane.

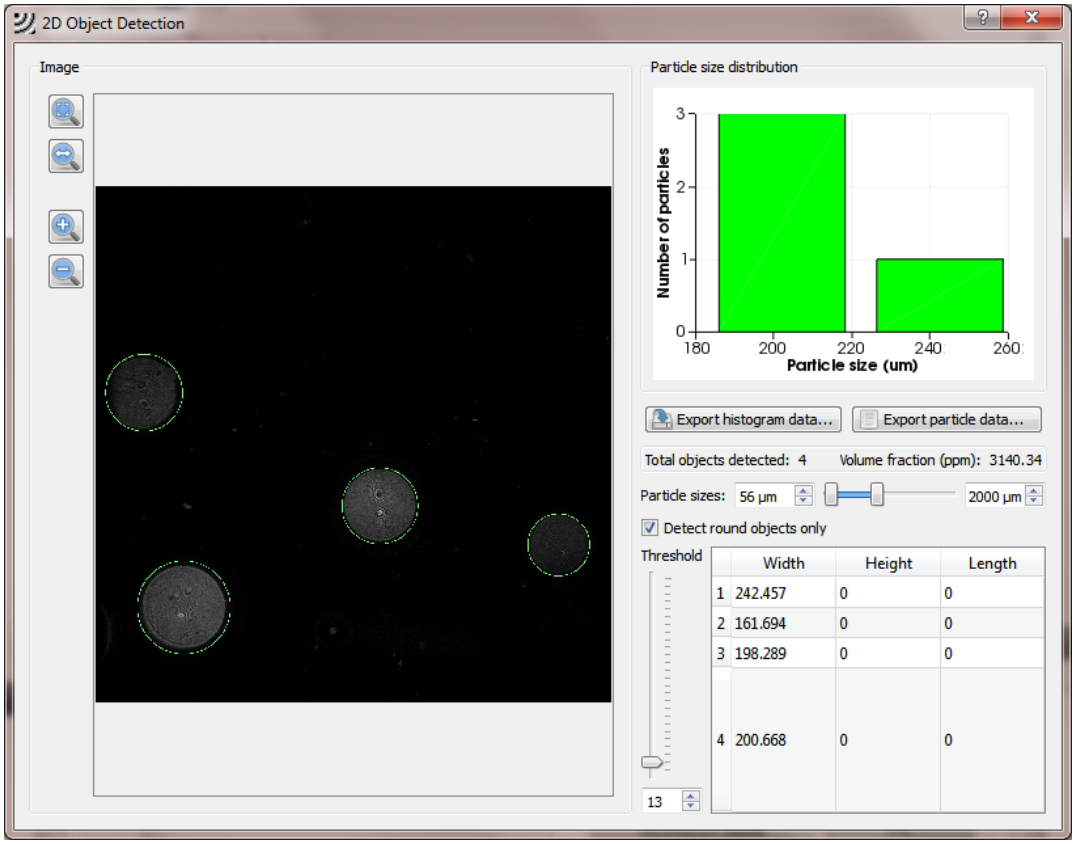
Slice controls:
Reset slices - resets the slice planes and changes zoom to the default value.
Measure on Red, Green, Blue - starts measure mode on the respective plane panel. Drag the measure tool ends with the mouse to adjust the positions. To exit the measure mode, click on the respective Measure button again.



Color map - show color-mapped slice views instead of grayscale views.

Dialogs

2D Object Detection



The 2D object detection dialog allows for the automated detection and measurements of objects in the reconstructed plane.

Parameters:
Particle sizes - minimum and maximum size of particles (in µm) to be detected.
Detect round objects only - select to detect only round objects.
Threshold - the intensity threshold of objects to be detected.

Results:
Table of all the detected particles. The Width and Height (in µm) for non round particles, or Width (diameter) for round particles is displayed in the table.

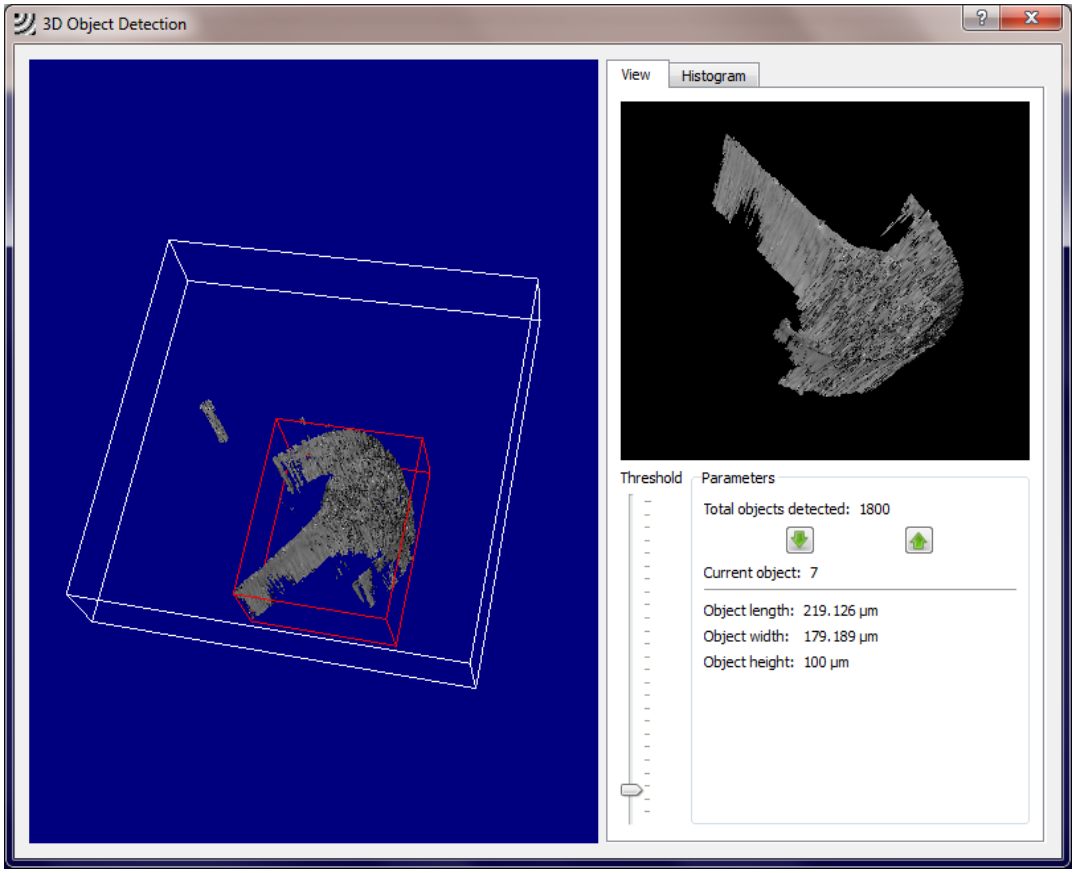
The detected particles are outlined in the image view. The control of the image view zoom is the same as in the results view for the reconstructions.

Total number of particles detected, and particle volume fraction in parts per million (ppm).

Particle size distribution histogram (based on Size or Volume of the particles).

Data export:
Export histogram data – exports the analyzed particle data and histogram to an MS Excel spreadsheet.
Export particle data – exports raw particle data (position and size of each detected particle) to an MS Excel spreadsheet.

3D Object Detection



The 3D Object Detection allows for the detection and measurement of objects in 3D space.

The currently detected object is shown with the red rectangular outline in the context of the volume. The detected object is shown in a separate magnified view and can be rotated, panned and zoomed.

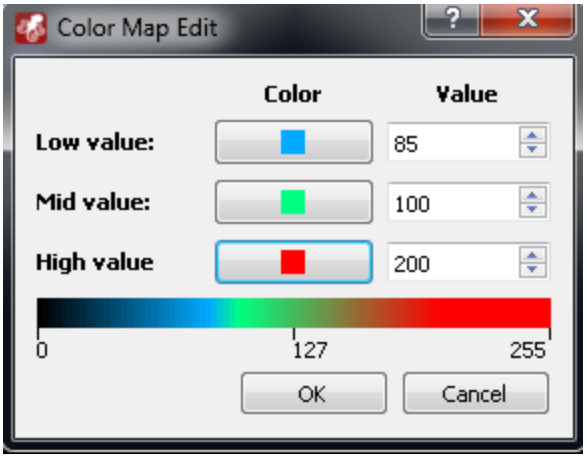
The size of the detected object in 3 dimensions is shown (in µm).

3D detection controls:
Threshold - adjust the threshold for the voxel intensity to be used as a parameter for the isosurface 3D plot.
Next/Previous object - selects next/previous object in the 3D view.
Histogram - allows for the plotting of particle size or volume distribution histogram for the detected objects. The largest dimension of each object is being used to calculate the size histogram.

Data export:
Export histogram data – exports the analyzed particle data and histogram to an MS Excel spreadsheet.
Export particle data – exports raw particle data (position and size of each detected particle) to an MS Excel spreadsheet.

Edit Color Map

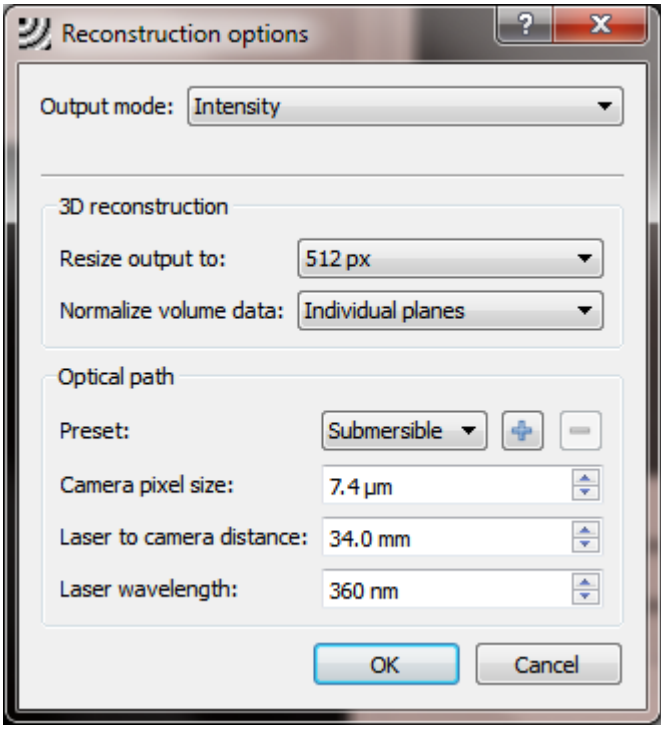
This dialog is being used to edit color maps that are used to apply artificial colors to data in the Reconstruction view, Volume view and Slice view. There are 3 data points in the color map: Low, Mid, and High. For each data point the color and scalar (from 0 to 255) can be set. The Preview of the color map is instantly updated and shown in the preview area.



Camera Options

The Camera Options dialog provide access to the low level options of the GigE Vision camera installed in the Resolution Optics Submersible or Desktop microscope. Note that the low level options may affect the image quality and reconstruction results, do not change the parameters unless you consult with Resolution Optics support first.

Reconstruction Options



The Reconstruction Options dialog is used to set hologram reconstruction mode, optical path configuration, and 3D reconstruction parameters.

Output mode - can be one of Intensity, Amplitude, or Phase

3D Reconstruction:
Resize output to - set to either downsize output for 3D volume reconstruction, or use the original reconstructed image size. Smaller image sizes improve performance and reduce memory usage.
Normalize volume data - Either normalize data for each reconstructed plane separately (0-255 intensity values), or re-

normalize the entire volume. Note that the whole volume normalization requires much larger memory buffer.

Optical path:
Camera pixel size - pixel size of the camera (in μm).
Laser to camera distance - distance (in mm) between the point source and the camera sensor.
Laser wavelength - wavelength of the laser light source (in nm).

Presets:
Presets are used for storing and quick recall of the optical path configurations. These can be used to store the optical configurations of the custom holographic setups, or experimental conditions.

The 2 standard presets: `Desktop` and `Submersible` correspond to the optimal configuration of the respective Resolution Optics microscopes.

Note that the standard presets cannot be deleted, and any modifications to these presets will not be stored – the default values will be restored upon the preset switch.

To create a new custom preset press the `Add preset (+)` button. The name of the preset can be changed upon creation. Any modifications to the preset will be stored when `OK` button is pressed.

To delete a custom preset, press the `Remove preset (-)` button.

Software Agreement

This software is proprietary to Resolution Optics Inc. and the company hereby grants the customer a nonexclusive license for its use. The Customer will not modify, adapt, translate, create derivative works, disassemble, decompile or reverse engineer the software provided. No title to or ownership of the software or intellectual property rights are transferred to the customer.