

4Deep

inwater imaging

Octopus Software User Guide

Version 1.6.0



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1 Introduction

The Octopus software has been developed to operate in conjunction with either 4Deep submersible or benchtop holographic microscopes. The 4Deep submersible microscopes are holographic microscopes which can operate while fully submerged in water. The desktop microscopes are portable benchtop instruments.

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 benchtop 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
- Counting, data and morphological analysis, quantitative phase analysis: many other applications

2 Quick Installation Guide

To install Octopus software on your computer

- Download the software from our website: <http://4-deep.com/software-downloads/>.
- Insert the HASP key supplied; Follow the onscreen instructions.
- Note that for fast hologram reconstructions, 4Deep software requires a CUDA-enabled NVIDIA graphics card to be installed in the computer. For the list of CUDA-enabled graphics chips, refer to <https://developer.nvidia.com/cuda-gpus>.
- If your NVIDIA drivers are not up to date, please update them at <http://www.nvidia.com/Download/index.aspx>.
- Install Octopus by running OctopusInstaller.exe and following the onscreen instructions. Selecting the default parameters should typically be acceptable for most installations.

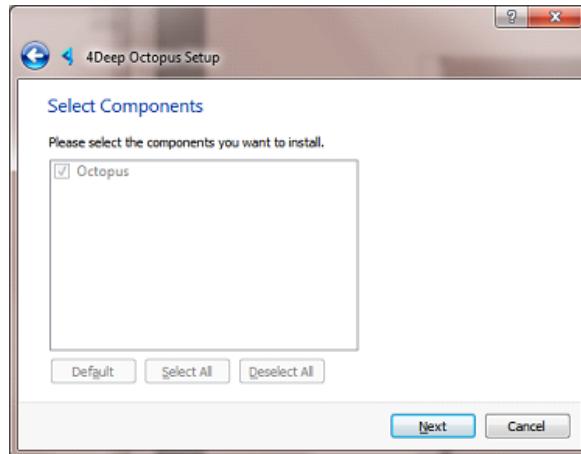


Figure 1: Octopus installer, selection of the installation package

2.1 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-> 4Deep-> Octopus](#). The Octopus software will start.

When started for the first time, Octopus offers to select the instrument profile, or create a new one. Click the preset in the drop down menu that corresponds to the instrument being used, as the instruments have different laser to camera distances and pixel sizes.

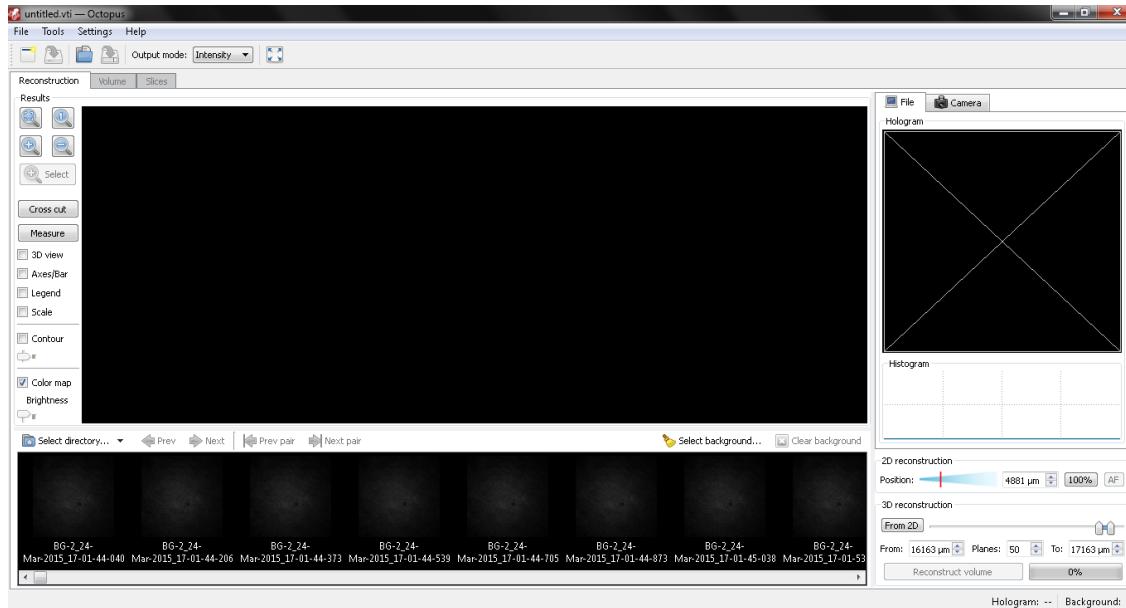


Figure 2: Octopus Software user interface after launch

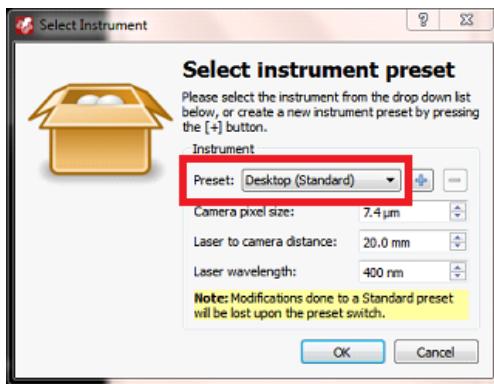


Figure 3: Select instrument preset dialog

3 A Brief Introduction to Hologram Reconstruction

3.1 The Hologram

The Octopus software works with the submersible or benchtop microscopes, and controls the camera to capture holograms.

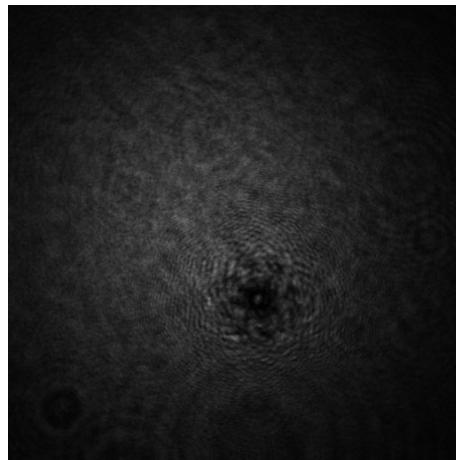
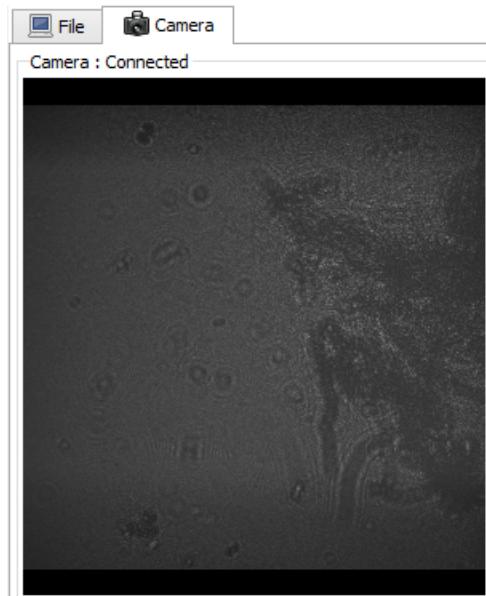


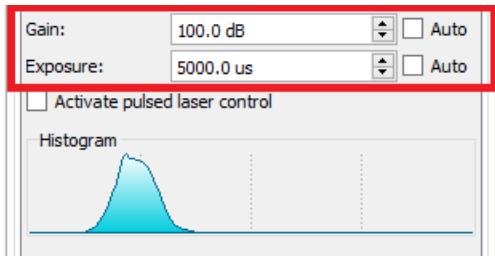
Figure 4: The hologram, captured here using the 4Deep Submersible microscope in the Atlantic Ocean

3.1.1 Getting a Hologram With Octopus

- Turn on the software, and connect the computer to a 4Deep microscope, and turn on the microscope. Connect to the camera by clicking the [Camera](#) view tab.



- Check the exposure level of the hologram using the [histogram](#) located just below the camera controls, and in the camera control, adjust the [Gain](#), [Exposure](#)



or [Pulse duration](#)

by clicking on the corresponding numbers and inputting new values, or by clicking the scroll up or down. Gain artificially increases the intensity of the camera signal, exposure is the amount of time the camera is collecting light, and pulse duration is the length of time the laser is illuminating the subject.

If the hologram is under exposed, there will be many black pixels, and the **histogram will be to the left**; increase the gain and exposure or pulse duration.

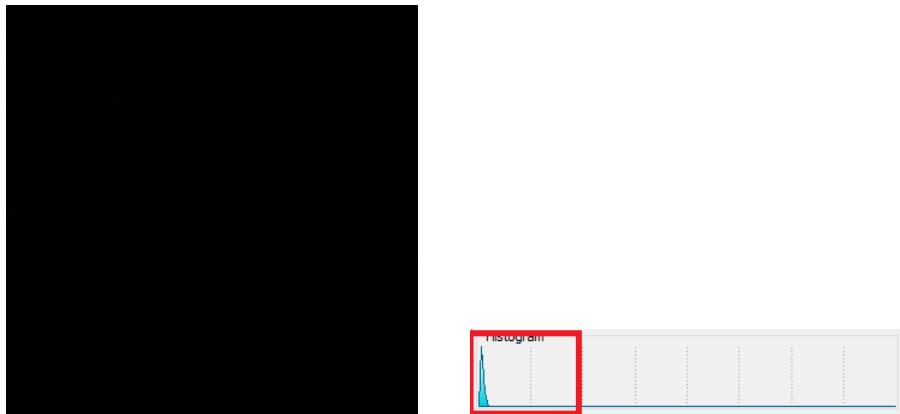


Figure 5: An under exposed hologram, and the histogram associated with under exposure.

If the hologram is over exposed, there will be many white pixels, and the **histogram will be to the right**; decrease the gain and exposure or pulse duration.

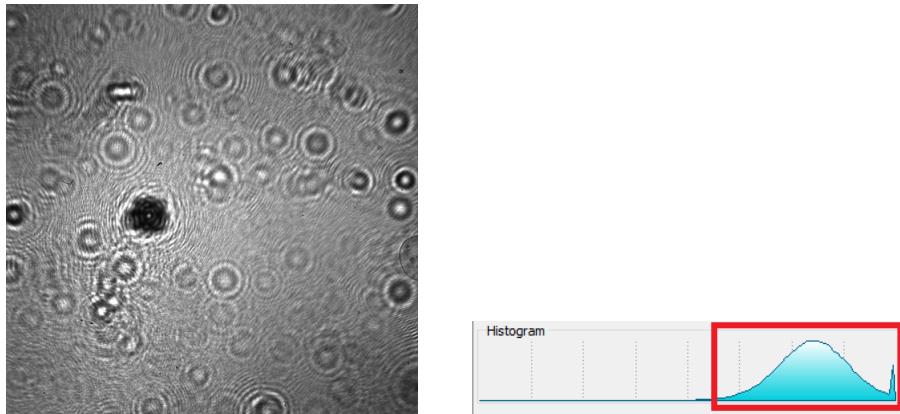


Figure 6: An over exposed hologram, and the histogram associated with over exposure.

A properly exposed hologram will have no white pixels side by side near the centre, and little black around the edges; the histogram will be slightly left of centre. The control of gain and exposure can be handled automatically by Octopus, by checking the **Auto** boxes for gain and exposure.

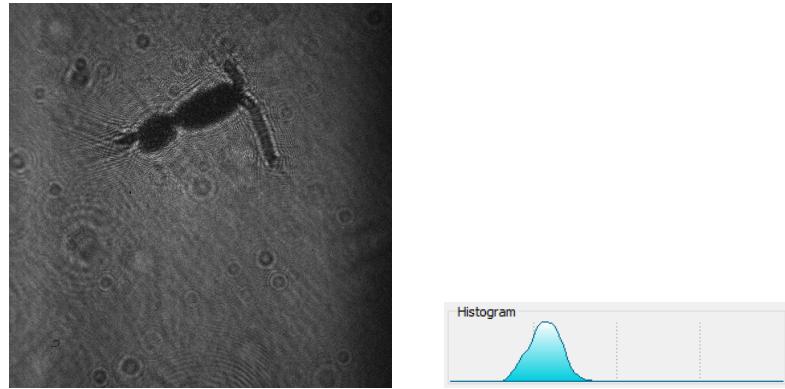


Figure 7: A properly exposed hologram, and the associated histogram.

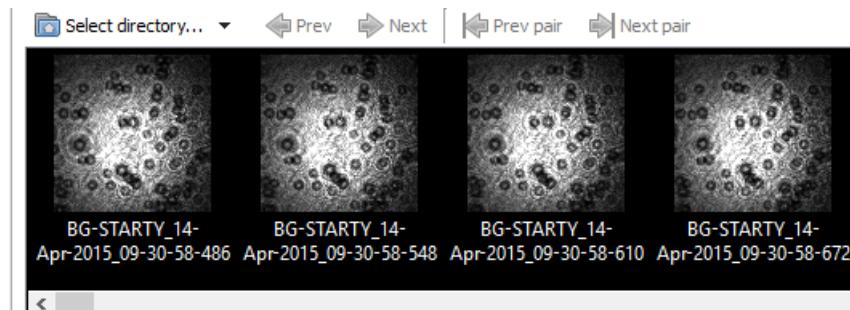
- Select the directory to save to , and give the file a name; each hologram will have the name as a prefix, accompanied by a time stamp.
- Select the camera controls, including frame rate, in frames per second.
- Select the **Frames in burst** to be less than 2, which is the **Continuous run** setting.
- Press the **Record** button to start and stop recording holograms.



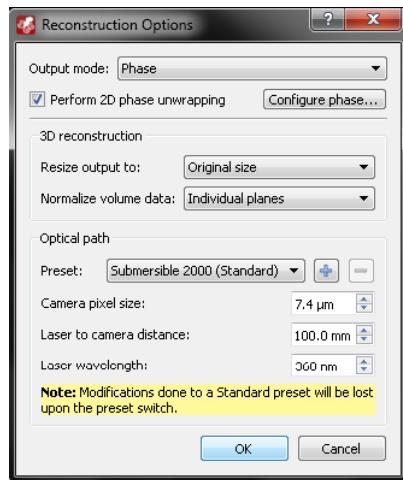
Greater detail of the camera controls can be found in Section 4, User Interface.

3.2 Reconstruct Holograms in Octopus

- Select the hologram to be reconstructed using the Reconstruct Holograms command, by drag and drop of selected holograms, or by clicking **Select directory...** and choosing the holograms from the file preview.



- Input the correct microscope settings in Reconstruction Options, by going to **Settings** in the Main Menu, and selecting from the **presets** in the drop down menu or inputting a customized setting.



- Select the **output mode** to be **Intensity** or **Phase** (more on phase reconstruction ahead in Section 3.4). Close the Reconstruction Options dialog to continue with reconstructions.

- Select a background hologram by clicking **Select background...**, if desired.

- To get the image in sharp focus, move along the Z-axis using the hot keys (**A D W X**, etc.), or by clicking on the **Position** cone and using the scroll wheel; the position from the point source is displayed with the numeric. Clicking on the numeric will give 1 micron steps along the Z-axis with the **Up/Down arrows**, or by clicking the scroll arrows. Auto Focus can be used by highlighting the area by a click and drag, and using the **AF** button.



- Move around in the XY-plane using the mouse scroll wheel to zoom, or the buttons to the left of the 2D reconstruction, and grab to pan with a click and hold of the scroll wheel.



- Move from one hologram to the next using hot keys **Alt+P** and **Alt+N**, or view successive holograms paired (as a compound reconstruction) with **Alt+X** and **Alt+E**; the same results come from the corresponding buttons above the file previews.



Greater detail of hologram reconstruction options can be found in Section 4, User Interface.

3.3 The Reconstruction

The reconstructed images can be further analyzed, saved, or assembled into the volume reconstructions based on the application. Octopus will reconstruct selected holograms automatically, and produce the resulting image of the 2D plane selected by **Position**.

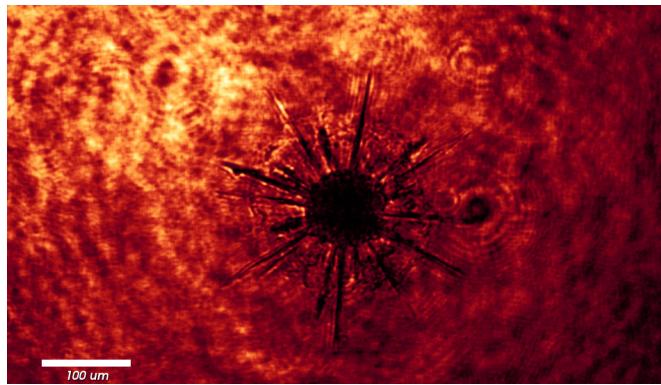


Figure 8: The reconstruction of the hologram in Figure 4

Figure 8 shows an image which has the sequential orange **Color map** applied, which changes the grey scale values to selected false colors; From the Main Menu, go to **Settings -> Edit color map**. The calibration **Bar** has been applied, when the box is checked, the bar provides a calibrated scale to the image in the lower left corner. The image can be reconstructed with background subtraction by selecting the background hologram; one with as few objects as possible, and from the same collection conditions works best.

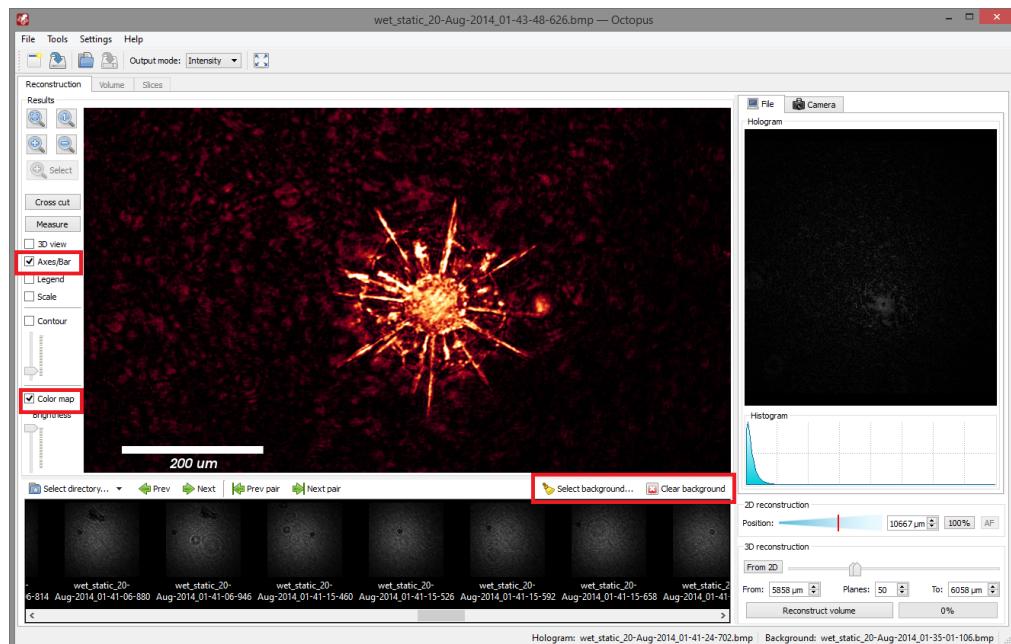
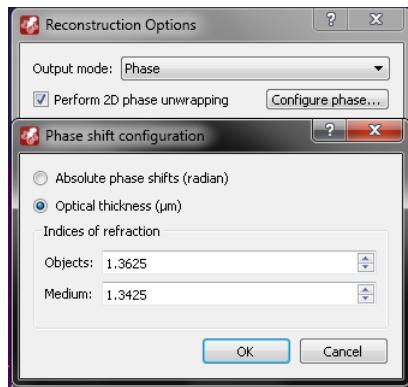


Figure 9: The reconstruction of the hologram in Figure 4, with background subtraction; Bar, Color map and Select background are highlighted

3.4 Quantitative Phase Information in Octopus

Reconstructions can be done using intensity, amplitude or phase, depending on the chosen **output mode**.

- In the **Settings** menu option, select Reconstruction Options, and select **phase** output from the drop down menu.



- Check the **Perform 2D phase unwrapping** box and click the **Configure phase...** button to toggle the Phase shift configuration dialog. Select the **Optical thickness (μm)** option and input the refractive indices for the media and objects; the refractive indices need to be accurate if the calculated measurements are to come out accurately. Lists of the refractive indices for common things can be found online: http://en.wikipedia.org/wiki/List_of_refractive_indices
- Close the dialogs and return to the reconstruction, which will look considerably different. The color map values are now in microns from the reconstruction plane, giving the optical thickness of the object.

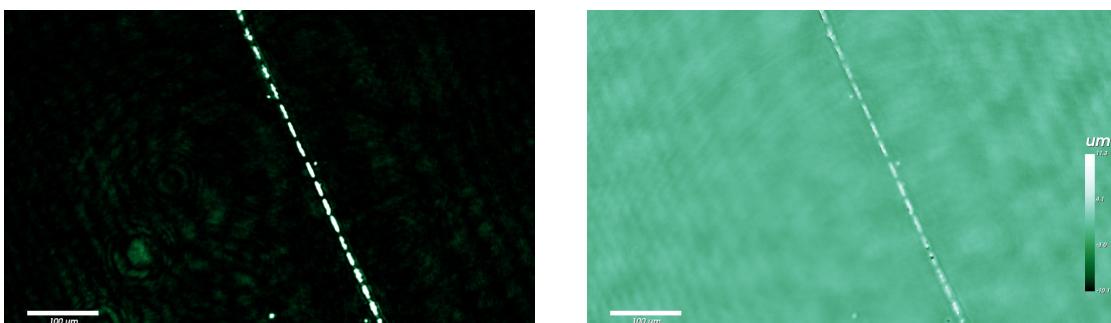
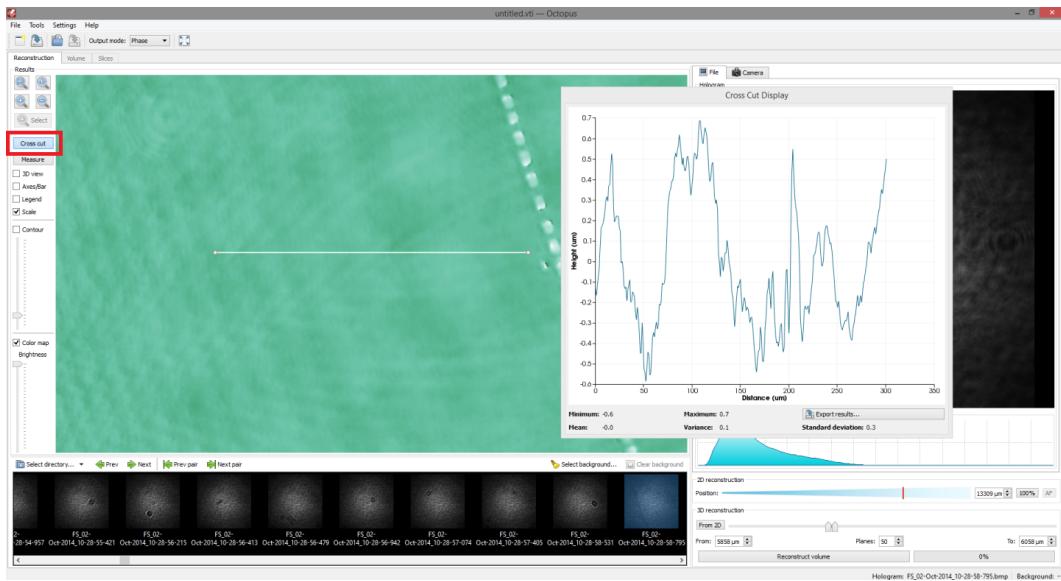


Figure 10: Reconstruction of an algae chain using intensity and background subtraction (left), and the same hologram represented in phase information; the scale on the right reads out in microns.

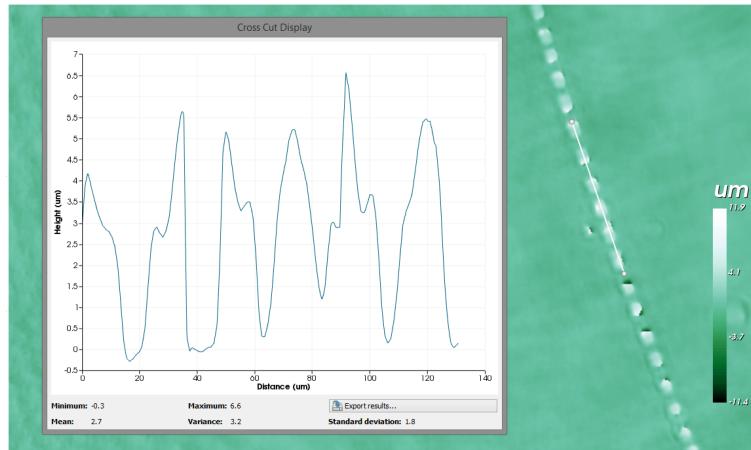
- Clicking on the **Scale** box on the left hand side of the screen will show the thickness of objects with a scale on the view.

An optimal way of using phase reconstructions is with the **Cross cut** tool.

- Click on the **Cross cut** button on the left hand side of the screen to toggle the Cross cut dialog. The Cross cut bar will appear on the reconstruction view.



- Move the Cross cut bar around by clicking and holding on to the barbells, or on to the centre of the bar. Move the dialog window by clicking on the top window bar. Select a place for the window that shows the cut and the results clearly.



Results of the Cross cut can be exported as an Excel or CSV file by clicking on the [Export results...](#) button in the dialog.

4 User Interface

4.1 Main Menu

4.1.1 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 a 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. The chosen holograms will be displayed in the image gallery preview bar. Holograms may be selected for reconstruction by a drag and drop of the holograms into the Octopus window.

Save Result Image - saves the current view of the 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.

4.1.2 Tools

2D Object Detection - opens the 2D Object Detection dialog. Refer to the dialog description (on page 26) for details.

3D Object Detection - opens the 3D Object Detection dialog. Refer to the dialog description (on page 27) for details.

4.1.3 Settings

Camera Options - opens the Camera Options dialog. Refer to the dialog description (on page 28) for details.

Edit Color map - opens the edit color map window. Refer to the dialog description (on page 28) for details.

Reconstruction Options - opens the Reconstruction Options dialog. Refer to the dialog description (on page 29) for details.

4.1.4 Help

About - shows information about the software.

User Guide - opens software User Guide.

Check for updates - Octopus will check for updates when prompted.

4.2 Tool Bar

 **Reconstruct Holograms** - Opens a file directory dialog to choose holograms for reconstruction; the chosen holograms will be displayed in the preview bar.

 **Save result image** - Saves the reconstruction window as a .png image; included in the image will be the **Legend**, **Scale**, **Measurement** and **Axes** or **Bar** when selected. This includes the saving the **3D view**, when selected.

 **Open volume file** - Opens a file directory dialog to choose a previously saved volume reconstruction file.

 **Save volume** - Saves the volume reconstruction.

The **Output mode** drop down menu selects the reconstruction output mode to intensity, amplitude (the root of intensity) and phase, the differences in the speed of light through the medium and objects.

 **Full screen** - Opens the 2D reconstruction window (or the 3D view) to the full screen, **F11** works as a hot key. In full screen, the position is changed with the hot keys. Press **Close** in the top left corner, **Esc** or **F11** to exit the full screen.

4.3 Main Window

4.3.1 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.

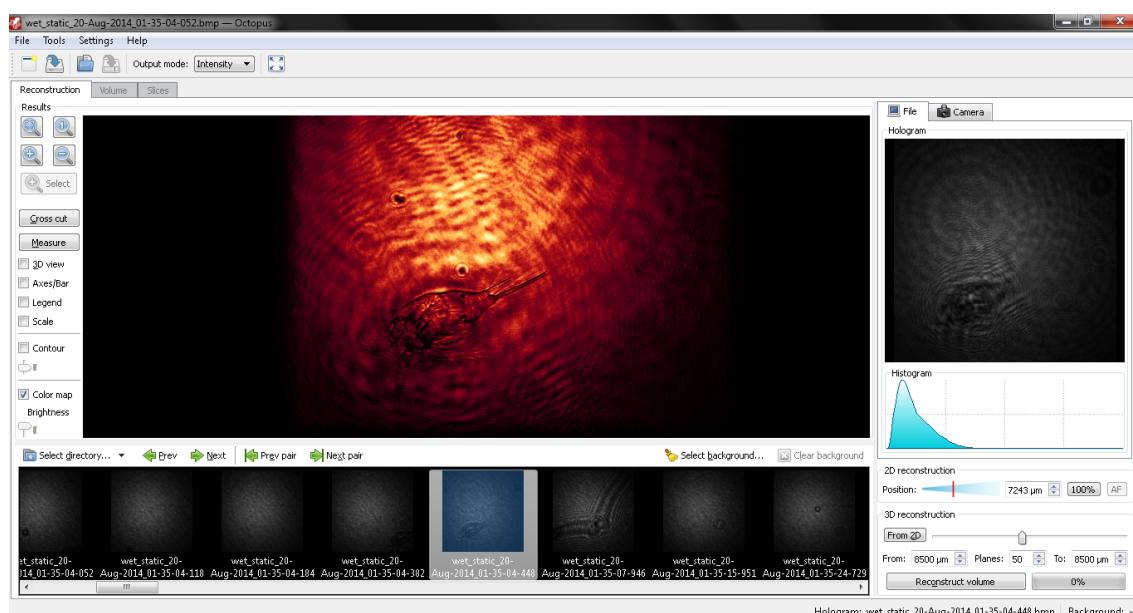


Figure 11: Reconstruction tab

Open a new directory of holograms with the **Select directory...** button, the resulting holograms will be displayed in the image gallery preview; the drop down menu will have the last five directories selected. To control what hologram is being reconstructed, use the **Next** and **Prev** buttons. These buttons select the next/previous hologram for reconstruction in the current folder. **Alt+N** and **Alt+P** perform the same function; the **left/right arrow** performs these functions when the image gallery is selected. Multiple holograms may be selected from the gallery, with pair wise background subtraction taking place.



Two holograms at a time may be chosen, with the second hologram chosen becoming the background. Holograms can be reconstructed in sequential pairs with the **Prev pair** and **Next pair** buttons, or with **Alt+E** and **Alt+X** respectively. The resulting compound hologram, where one hologram is subtracted from another (*Background Subtraction*), will be shown in the preview of the loaded hologram.

 Select background... Clear background

Select background... button allows to select the background hologram that will be subtracted from the current hologram prior to reconstruction; **Alt+B** does the same. 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.

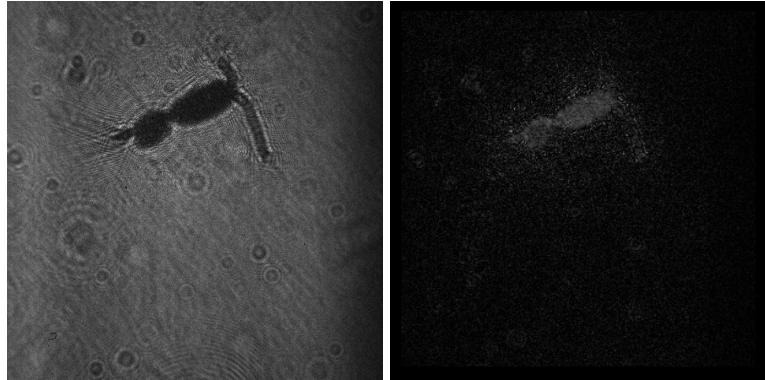
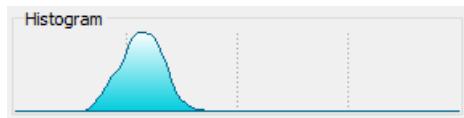


Figure 12: A hologram (left), and the compound hologram (right) after background subtraction

Clear background button, or **Alt+L**, removes the background hologram and reconstructs the hologram with no background subtraction.

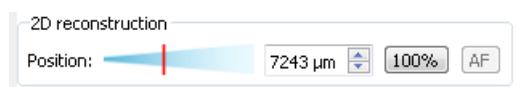
4.3.2 Histogram



The histogram gives the user a visual cue to the contrast found in the hologram. A spike at the start (left) of the horizontal scale indicates an under exposed hologram, and a spike at the end (right) indicates over exposure of the hologram. The histogram represented both here and in Figure 11 show smooth peaks, with no spikes at the ends, and can be considered histograms of good holograms.

Over and under exposure of holograms leads to the loss of information, and subsequent image quality. When collecting holograms using 4Deep microscopes, adjust the camera controls so that the holograms saved are not over or under exposed.

4.3.3 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, with the scroll wheel, or the position input field. Change the position and hologram will be automatically reconstructed at this new position.

There are several ways to control the Z-position for the reconstructions:

- With position slider, drag the slider and update the position. It's possible to use **page up/down** keys, when slider is selected. This will advance the position by laser to camera distance / 100. When arrow **up/down** keys are used, position will change by laser to camera distance / 1000.
- Global keys **W** and **X** perform the same action as the slider or **page up/down** keys, whereas global keys **A** and **D** perform the same action as arrow **up/down** keys. When global keys are used, there is no need to select the position slider first.
- With position spinbox activated, the numerical value for the reconstruction position can be directly entered. In addition, **page up/down** keys will advance the position by 10 μm , whereas arrow **up/down** keys will advance the position by 1 μm . When keys are pressed and held, the position will change with acceleration – the longer the key is pressed, the faster the value will change.

100% button toggles a drop down slider to use digital zoom, up to 200%. Reconstructions are cropped in this process, so objects at the edges of the original reconstructions may not be included in the zoomed reconstruction.

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:

- Select the object of interest in the Result View by left-clicking and dragging with mouse.

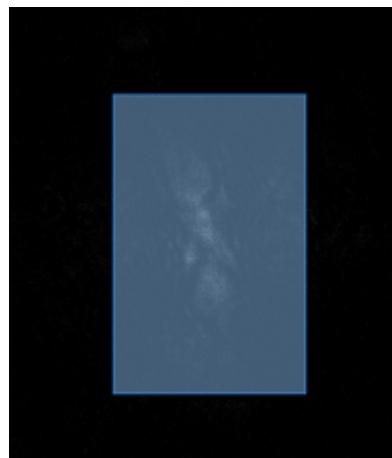


Figure 13: Object out of focus

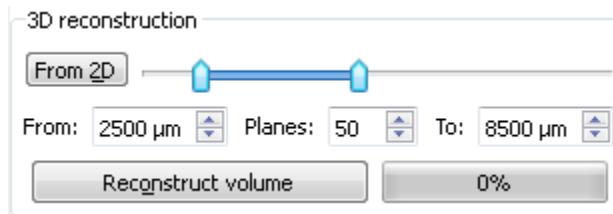
- Press the **AF** button, or press **Ctrl+A** on keyboard.
- The object will come into the optimal focus.



Figure 14: Object from above in focus

4.3.4 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: $(\text{To} - \text{From}) / (\text{Number of Planes} - 1)$.

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.

4.3.5 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 the previously selected region.

4.3.6 Cross Cut

The **Cross cut** button produces a line on the reconstructed image and a graph of the pixel intensity values along the line when in intensity or amplitude output mode, and values of phase shifts (in radians) or optical thickness (in microns) when in phase output (depending on phase configuration). Click and drag the bollards to move the line. Statistics are presented, and the cut data can be exported to MS Excel file or CSV text file.

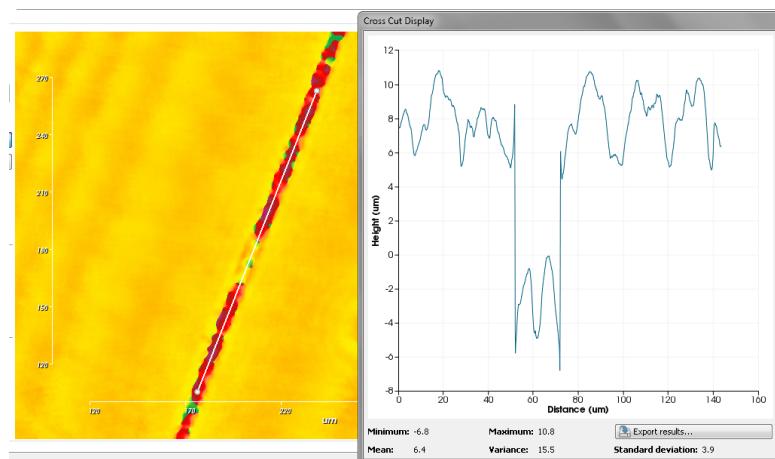


Figure 15: Crosscut function

4.3.7 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. **Alt+M** works as a hot key.

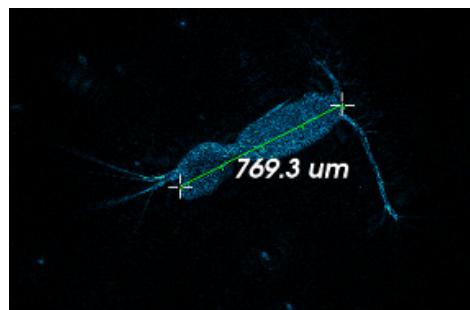


Figure 16: Measuring a reconstructed image

4.3.8 3D view

Checking the 3D view box will load the display window into a 3D view. Reconstructions run slower in the 3D view; the vertical height displayed is a visual representation of intensity, unless phase reconstructions are

performed and refractive indices are set, then the 3D view is representative of the optical thickness of the object.

4.3.9 Legend, Axes/Bar and Scale

Checking the boxes will produce the legend on the upper right of the reconstructed image, the axes along the lower and left hand side of the image, with a second click giving the calibration bar at the lower left, and the intensity scale in the bottom right corner. Legend, axes, bar and scale are saved on the result images.

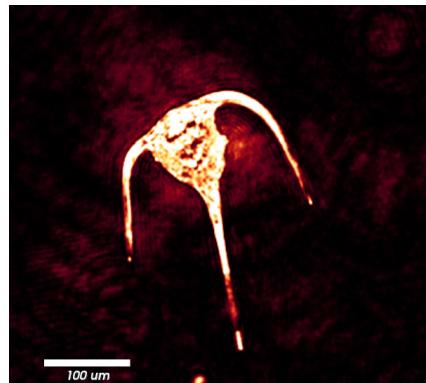


Figure 17: Calibrated Bar for reconstructed images

4.3.10 Contour

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

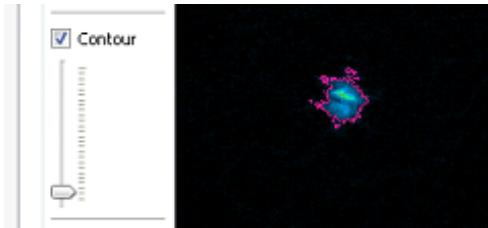


Figure 18: Contour selection tool

4.3.11 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; Refer to the dialog description (on page 28) for details.

4.3.12 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 4Deep submersible and benchtop microscopes are supported out of the box. For support of the 3rd party cameras in custom holographic setups contact a 4Deep representative or support line.

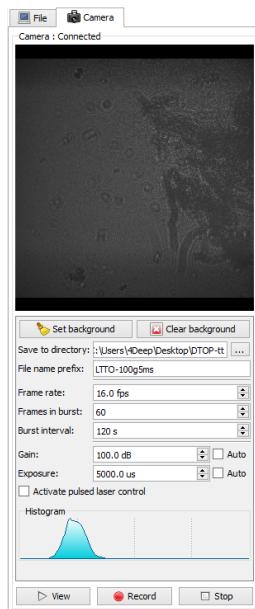
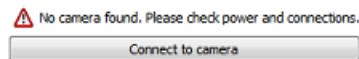


Figure 19: Camera view and controls

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. 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:

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:	Development\Octopus\debug
File name prefix:	test
Frame rate:	10.000 fps
Frames in burst:	10
Burst interval:	60 s

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.

Activate laser pulse control - the submersible microscopes from 4Deep have the ability to pulse the laser, which is controlled by the user when the option is selected. The laser will then pulse once during the exposure of the camera; in this configuration, exposure control does not change the captured hologram, only the gain and pulse duration settings will control the intensity of the hologram.

Pulsed laser control is important when the user needs to capture holograms of fast moving objects, or use the submersible microscope in flowing water conditions or towing.

Pulse duration - user control over the length of the pulse generated. Pulse durations above 6 microseconds tend to give overexposed holograms from the camera. Use a pulse duration as short as possible while still illuminating the sample; inspect the holograms from the camera feed for pixel over/under saturation, and check the histogram. The Pulse can be set automatically based on the gain by clicking the **Auto** check box.

Histogram - indicator for exposure of the holograms coming from the camera feed. Adjust Gain, Exposure or Pulse duration to smooth peaks or rough edges in the histogram.

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.

Note that camera can also be controlled using special internet-based protocol, described in the section “**Remote control**” below (on page 31).

4.3.13 Volume Tab

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

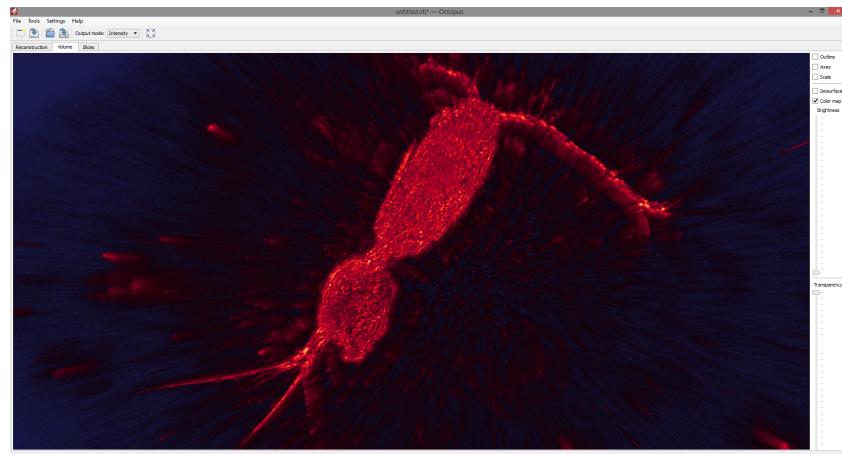


Figure 20: Volume reconstruction

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.

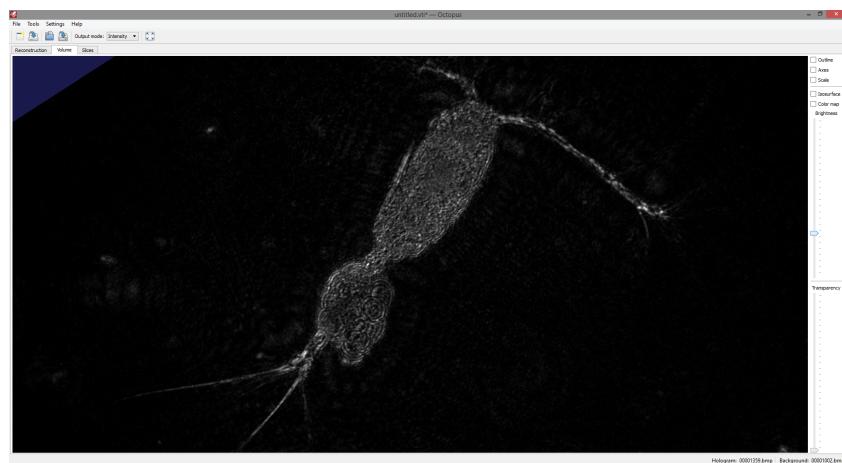


Figure 21: Volume view, grayscale mode, 0% transparency

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%.

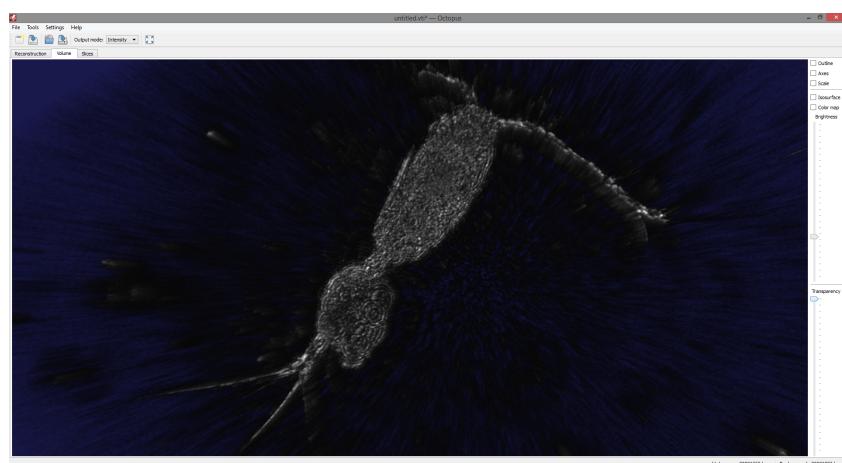


Figure 22: Volume view, grayscale mode, 100% transparency

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.

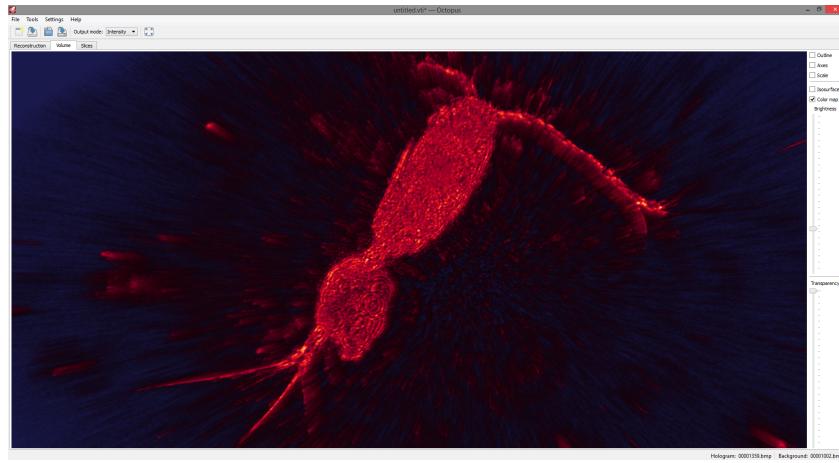


Figure 23: Volume view, colormap mode, 100% transparency

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.

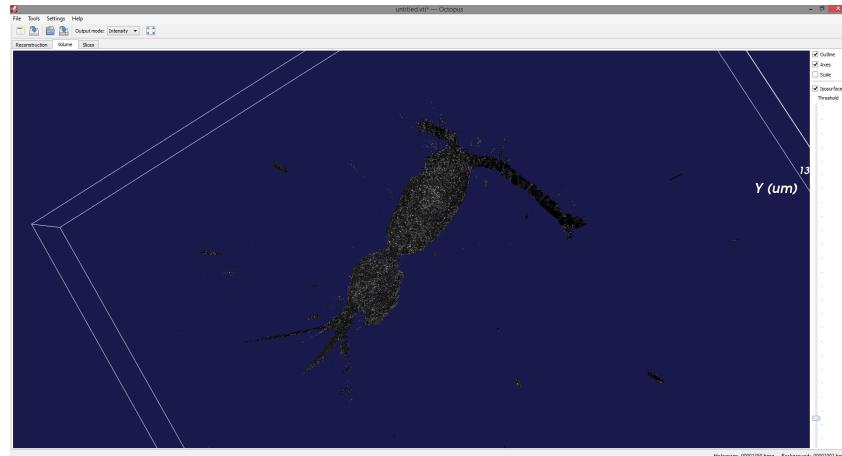


Figure 24: Volume view, isosurface mode

Additionally, **Outline** produces a bounding box around the volume, **Axes** shows the three axes, and **Scale** produces the intensity scale bar similar to 2D reconstructions. All the options selected in the Volume view will be saved upon Octopus exit and recalled on the next launch.

4.3.14 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.

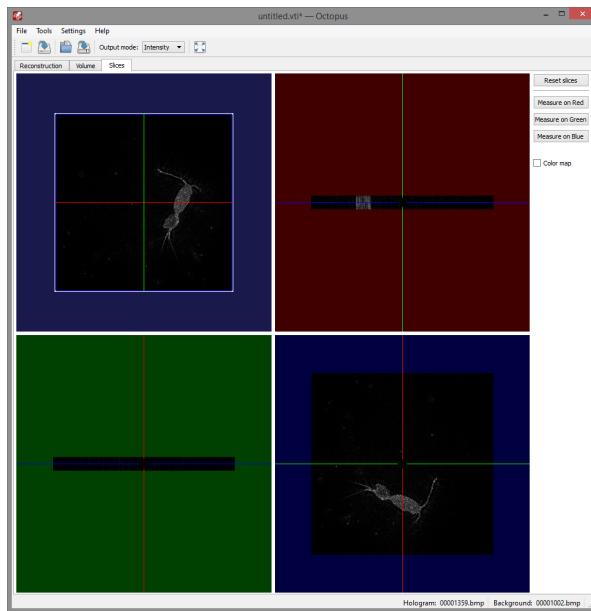


Figure 25: View of slices tab

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.

4.4 Quantitative Phase Contrast Imaging

One advantage of holography is that coherent light can be used to measure optical path length differences through translucent objects with great precision. With knowledge of the refractive indices of the media and the object, Octopus can unwrap a 2D phase reconstruction to reveal the thickness of the objects by utilizing the optical path differences. The 3D view option is best utilized with this method.

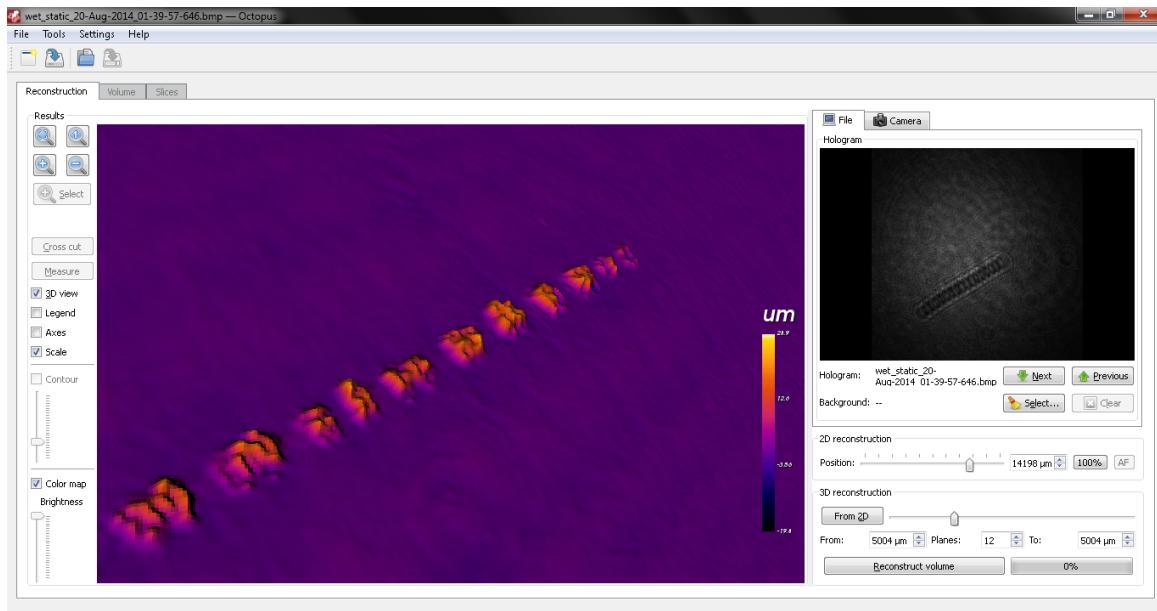


Figure 26: 3D view of quantitative phase contrast imaging in Octopus

To begin a reconstruction representative of the thickness of the object, select a hologram, and from the Reconstruction Options dialog, set the **Output mode** to Phase, then choose **2D phase unwrapping** and click the **Configure phase...** button, which toggles the Phase shift configuration dialog. The user can choose **Absolute phase shifts**, which gives the phase shift values in radians, or **Optical thickness**, where the user inputs the refractive index of the media and the object. The refractive indices need to be accurate if the calculated measurements are to come out accurately. Lists of the refractive indices for common things can be found on line: http://en.wikipedia.org/wiki/List_of_refractive_indices

Input the indices and the reconstructions will give measurements of the thickness of the objects viewed. With the **Scale** selected in this output, the colours indicate thickness, with the scale corresponding to the thickness of the object in microns.

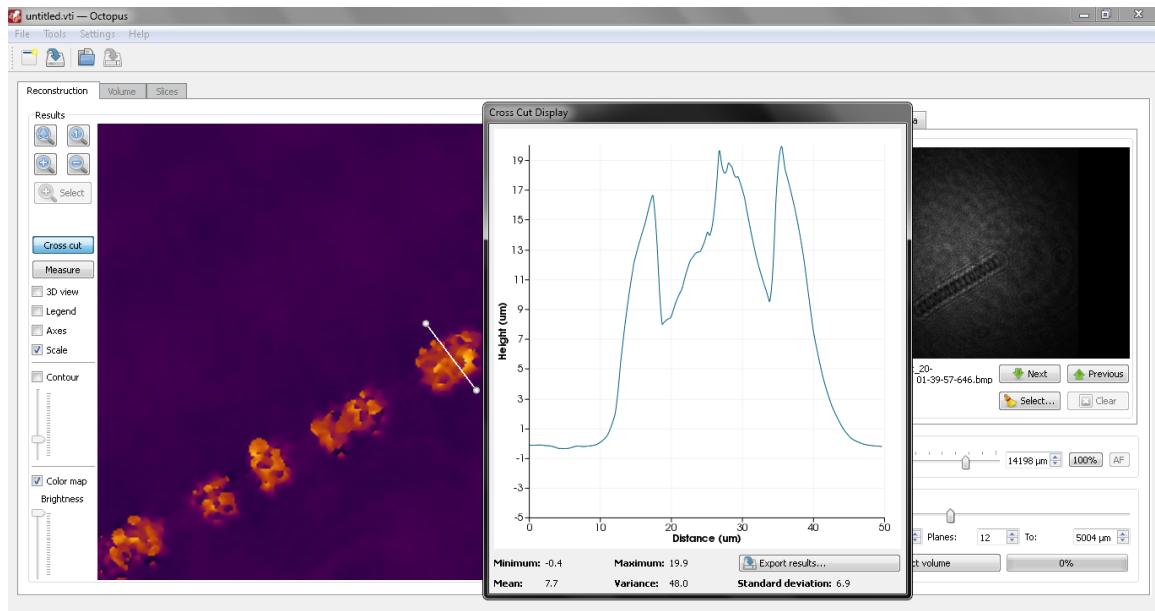


Figure 27: Crosscut of quantitative phase contrast imaging in Octopus

Now the **Cross cut** button gives dimensions in microns through the object selected. Along the x-axis, the length along the cut is displayed, and the y-axis is the thickness in microns along the cut.

4.5 Dialogs

4.5.1 2D Object Detection

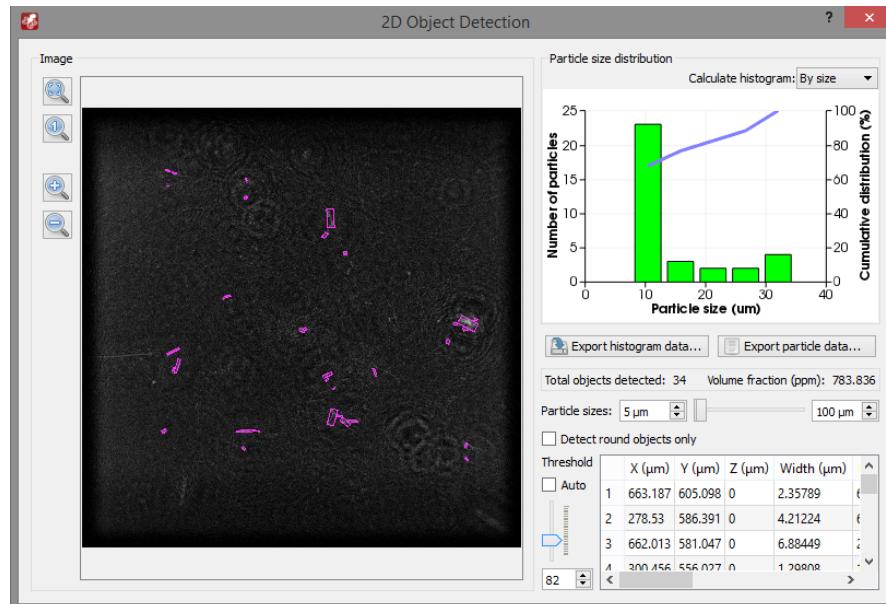


Figure 28: 2D object detection dialog

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

Parameters:

These parameters are the same as those found in 4Deep particle counting software, Swordfish. Analysis of 2D reconstructions

Particle sizes - minimum and maximum size of particles (in μm) to be detected.

Detect round objects only - select to detect only round objects.

Threshold/Auto 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; found on the left side of the dialog.

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 or CSV text file.

Export particle data – exports raw particle data (position and size of each detected particle) to an MS Excel spreadsheet or CSV text file.

4.5.2 3D Object Detection

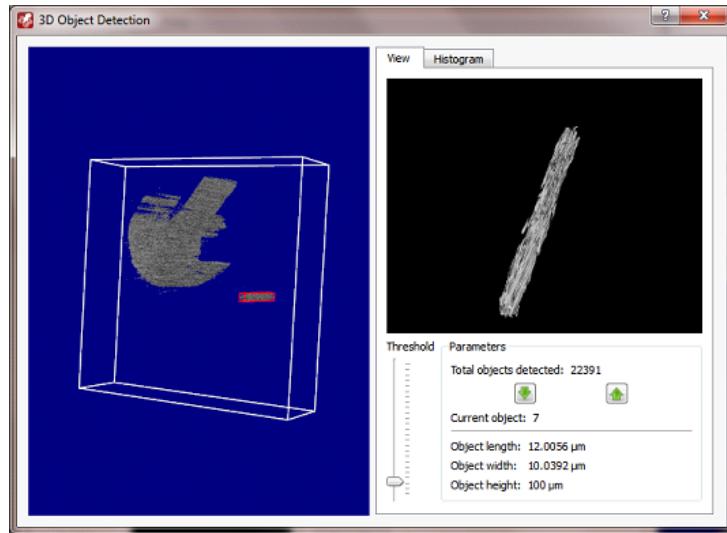


Figure 29: 3D object detection dialog

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 or CSV text file.

Export particle data – exports raw particle data (position and size of each detected particle) to an MS Excel spreadsheet or CSV text file.

4.5.3 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 editable data points in the color map. For each data point the color and scalar (from 0 to 255) can be set. Select the data point, what color you would like, and select the point on the scalar you want the color to represent. There are several data points on each palette which can be modified, and they can be added or subtracted with the + and - buttons on the lower left of the dialog. The end data points representing 0 and 255 cannot be moved, and are fixed points; the colors of the end data points may be changed. The preview of the color map is instantly updated and shown in the preview area.

There are several preset **Palettes** the user can choose from, depending on the object qualities which are to be highlighted. Customized palettes can be modified and saved to the list.

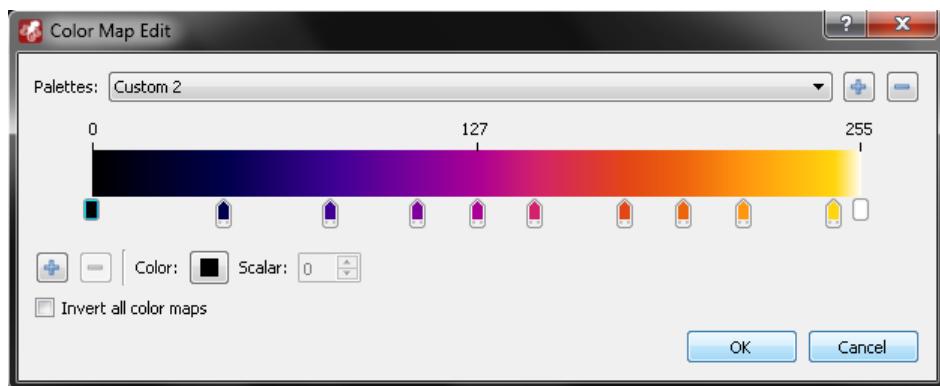


Figure 30: Color map edit dialog

4.5.4 Camera Options

The Camera Options dialog provide access to the low level options of the GigE Vision camera installed in the 4Deep submersible or benchtop microscope. Note that the low level options may affect the image quality and reconstruction results, do not change the parameters unless you consult with 4Deep support first.

4.5.5 Reconstruction Options

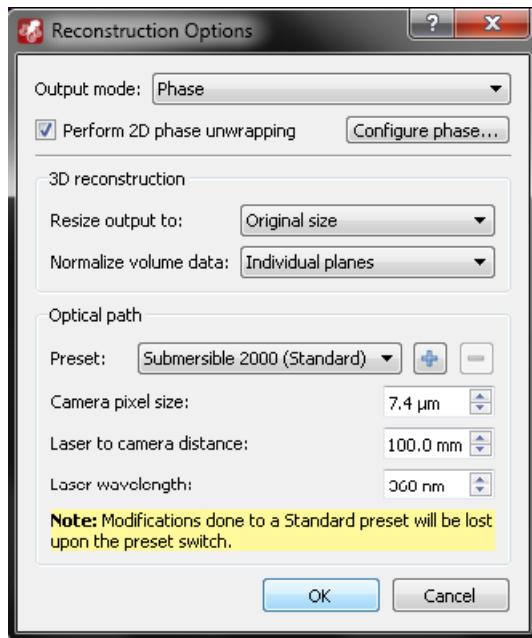


Figure 31: Reconstruction Options dialog

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.

2D phase unwrapping - In Phase output mode, the user can select to have the phase unwrapped on the reconstruction. This will result in slower reconstructions, and give information on the optical properties of the objects.

Configure phase - Toggles the Phase shift configuration dialog.

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 - relative 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 4 standard presets: **Desktop**, **Submersible**, **Submersible 2k** and **Cuvette** correspond to the optimal configuration of the respective 4Deep 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.

4.5.6 Phase shift configuration

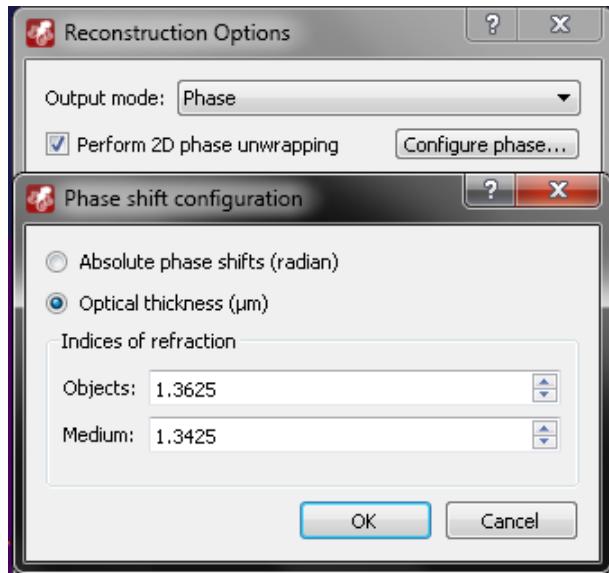


Figure 32: Phase shift configuration dialog

With Phase output selected, reconstructions can be wrapped or unwrapped; the output is controlled by the Phase shift configurations dialog, toggled from the Reconstruction options dialog. If the objects investigated are small (a few microns), the phase need not be unwrapped, increasing the speed of reconstructions.

Absolute phase shifts gives the phase shift value (relative to the media) in radians.

Optical thickness calculates the thickness of translucent objects according to the user input of refractive indices. Input the refractive index of the objects in **Objects**, and likewise with the medium in **Medium**. The calculated thickness of the objects is displayed with the **Scale** or **Cross cut** commands.

4.6 Hot Key Guide

Selected controls of Octopus have hot keys for quick access to commands.

Hot Key	Command
Ctrl+R	Reconstruct Hologram - opens directory dialog
Alt+D	Opens directory dialog
Alt+B	Opens directory to choose background hologram
Alt+L	Clears the selected background hologram
A	Moves the 2D reconstruction position down, or closer to the point source
D	Moves the 2D reconstruction position up, or closer to the camera
W	Moves 2D reconstruction position up by 10 x A
X	Moves 2D reconstruction position down by 10 x B
Page Up	Same as W , when the Position cone is selected
Page Down	Same as X , when the Position cone is selected
Up arrow	As D , with the Position cone selected; moves 1 μm up with numeric 2D position selected
Down arrow	As A , with the Position cone selected; moves 1 μm down with numerical selected
Right arrow	Selects the next hologram when the image gallery preview is selected
Left arrow	Selects the previous hologram when the image gallery preview is selected
Alt+X	Selects the next successive pair of holograms for reconstruction
Alt+E	Selects the previous pair of holograms for reconstruction
Alt+N	Selects the next hologram for reconstruction
Alt+P	Selects the previous hologram for reconstruction
F7	Toggle Reconstruction Options Dialog
F11	Toggle Full screen view
Ctrl+Z	Toggle digital zoom drop down
Ctrl+A	Auto focus when an area is selected
Ctrl+F	Full reconstruction view
Ctrl+1	One to one pixel view
Ctrl++	Zoom in to reconstruction
Ctrl+-	Zoom out of reconstruction
Ctrl+E	Zoom to view of selected area
Alt+C	Cross cut command
Alt+M	Measure command
Alt+3	Toggles 3D view
Ctrl+I	Saves reconstructed image
Alt+2	Reconstructs 3D from current 2D
Alt+O	3D reconstruction command
Ctrl+O	Open 3D volume
Ctrl+S	Save 3D volume
Ctrl+M	Toggle Edit colour map dialog
Ctrl+P	Print reconstructed image
Ctrl+Q	Quit Octopus
F1	Opens User Guide

5 Remote Control

Certain features of the Octopus software can be controlled remotely, using the special Internet-based protocol. The remote control allows you to change Octopus parameters from the same or different computer anywhere on the Internet. The remote control of the software operation can be performed using the supplied "Camera Remote Control" utility, or implemented in the 3d party software.

To implement the remote control in your software, you need to be able to connect, read, and write data from/to a TCP socket. The remote control is done by sending and receiving ASCII text strings through a specific TCP port. By default, all communication is happening on TCP port 1975. When Octopus starts, it launches TCP/IP server and waits for the incoming text commands. Note that you need to use the correct

IP address to connect to the TCP/IP server. If your computer has multiple network interfaces, try them all if first connection attempt fails. If you try to connect from the external network, make sure that port 1975 is forwarded by NAT to the machine that runs Octopus on the internal network. Make sure port 1975 is not blocked by your firewall.

All remote commands have the same structure:

COMMAND_NAME Value\n

Where COMMAND_NAME is the name of the command to be sent/received. Value (optional) – is the value to be sent together with the command. Value is separated from the command name by a space character. Each command-value string is terminated by a new line (“\n”) character.

After Octopus software processes the incoming command, it attempts to change the respective software feature or option (for example camera recording state, or exposure value). For every valid received command, Octopus will send a reply. Reply has the same command name as an incoming command, with “ACK_” prepended to the command name.

For example:

FRAME_RATE 12\n

requests Octopus to set camera frame rate to 12 frames per second. When frame rate is successfully set, Octopus replies with

ACK_FRAME_RATE 12\n

Do not assume that every command you send to Octopus will be correctly processed. Wait for a respective ACK_ reply and take the value from the reply as a new valid value. If the value or option cannot be set, Octopus will reply with the old valid value. For example, if we attempt to set camera burst interval to the invalid, negative value:

BURST_INTERVAL -15\n

Octopus will not update the burst interval, and will reply with the current, valid interval:

ACK_BURST_INTERVAL 60\n

Below is the list of remote commands with short descriptions:

ACTIVATE\n

No values. Activates the camera.

DEACTIVATE\n

No values. Deactivates the camera, stops acquisition or recording.

SYNC\n

No values. Requests Octopus to send current camera parameters (image directory, frame rate, burst interval, etc). All parameters will be sent as ACK_ replies. At the end of all the camera replies, ACK_SYNC reply will be sent.

VIEW\n

No values. Activates camera view mode. Replies with the timestamp when view mode has been activated. Timestamp is in the POSIX format – number of milliseconds since midnight, Jan 1 1970, UTC.

RECORD\n

No values. Activates camera record mode. Replies with the timestamp when record mode has been activated. Timestamp is in the POSIX format – number of milliseconds since midnight, Jan 1 1970, UTC.

STOP\n

No values. Stops the camera view/record mode. Replies with the timestamp when stop has been activated. Timestamp is in the POSIX format – number of milliseconds since midnight, Jan 1 1970, UTC.

IMAGE_DIRECTORY Val\n

Sets the current image directory for storing images recorded by the camera. Value is an absolute path to the valid directory where images will be stored.

IMAGE_PREFIX Val\n

Sets the prefix of image files. Value is a string that will be prepended to all image file names recorded by the camera.

FRAME_RATE Val\n

Sets camera frame rate. Value is frame rate in frames per second (floating point).

BURST_NUMBER Val\n

Sets camera burst frame number. Value is a number of frames in the burst of frames recorded by the camera (integer). If burst number is set to 1, continuous recording will be performed.

BURST_INTERVAL Val\n

Sets camera burst interval. Value is an interval in seconds between the bursts of frames recorded by the camera (integer).

GAIN Val\n

Sets camera gain. Value is a camera gain (usually in dB, depends on the camera model) (floating point).

AUTO_GAIN Val\n

Sets camera auto gain on or off. If value=0, auto gain is off, if value=1, auto gain is on (integer).

EXPOSURE Val\n

Sets camera exposure. Value is a camera exposure (usually in μ s, depends on the camera model) (floating point).

AUTO_EXPOSURE Val\n

Sets camera auto exposure on or off. If value=0, auto exposure is off, if value=1, auto exposure is on (integer).

ACTIVATE_PULSED Val\n

Activates or disables pulsed laser mode, parameters are 0 and 1.

STROBE Val\n

Manual control of strobe duration, parameter is strobe duration in μ s.

AUTO_STROBE Val\n

Parameter 1 or 0. Turns autostrobe off or on. Autostrobe is an algorithm to automatically control strobe (laser pulse) duration and gain based on histogram of received camera image.

DISTANCE_FROM Val\n

Distance from reconstruction position (in μ m) for 3D reconstruction.

DISTANCE_TO Val\n

Distance to reconstruction position (in μ m) for 3D reconstruction.

PLANES_COUNT Val\n

Number of planes to reconstruct for 3D reconstruction.

RECONSTRUCTION_POSITION Val\n

Reconstruction position (in μ m) for 2D reconstruction.

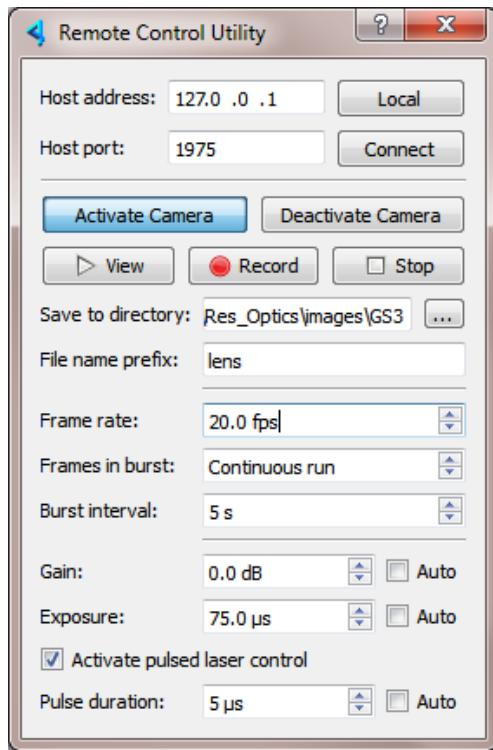


Figure 33: Camera Remote Control Utility

The convenience of Camera Remote Control utility can be downloaded and used to control Octopus from the local or external network. The utility implements the same remote control protocol described above. Make sure you are connecting to the correct host IP address – the address of the computer that runs Octopus software.

6 Appendix

6.1 Principle of Operation

The Octopus software works with the submersible or benchtop microscopes, which operate on the principles of holography to image a volume in magnification. A 405 nm laser is focused on an aperture 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.

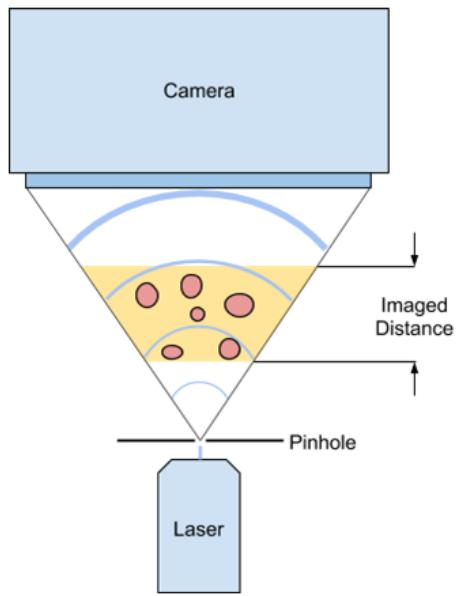


Figure 34: The basic principle of digital in-line holographic imaging

6.2 The Advantages of Holographic Microscopy

The images obtained from holographic microscopy, such as Figure 9, can be compared to that of dark field microscopy, except that the collection of the images requires no constraints on the media or samples. The field of view for traditional optical microscopes using lenses is typically a few microns; with holography, a larger field of view, up to a couple of centimeters, allows for more dynamic experimental conditions.

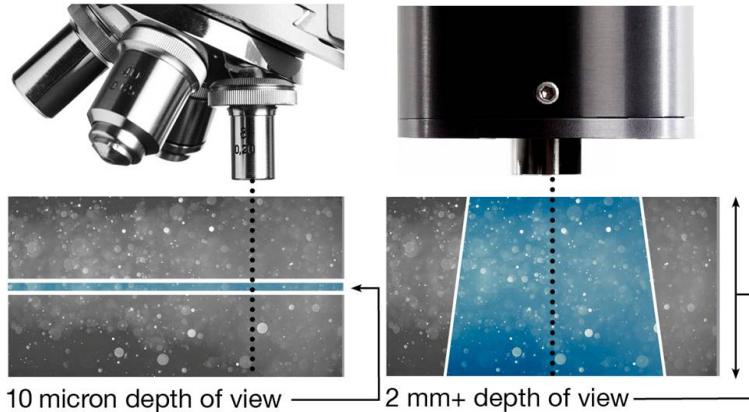


Figure 35: Field of view comparison of lens based microscope (left) and holographic microscope (right)

No need to stain or physically constrain samples with holography; the large field of view gives free movement of microscopic organisms and particles, and allows for the field deployable Submersible microscope from 4Deep, for real time, *in-situ* imaging.

Along with the spatial information providing the images in the reconstructions, holograms also contain the phase information; the differences in the speed of light passing through the objects is provided by this information, giving Quantitative Phase Imaging (QPI) capabilities to Octopus. The measurements of the

thickness of objects obtained from phase information can be very precise, as the phase information exists well below the wavelength of the light.

Holographic microscopes from 4Deep work simultaneously as a QPI microscope and as a dark field microscope, and gather information from a macroscopic field of view with each frame. With frame rates of 15 fps or more, and frame exposures down to 1 microsecond on some models, the holographic microscopes of 4Deep offer 4D imaging capabilities like no other microscopes.

7 Software Agreement

This software is proprietary to 4Deep inwater imaging 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.

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