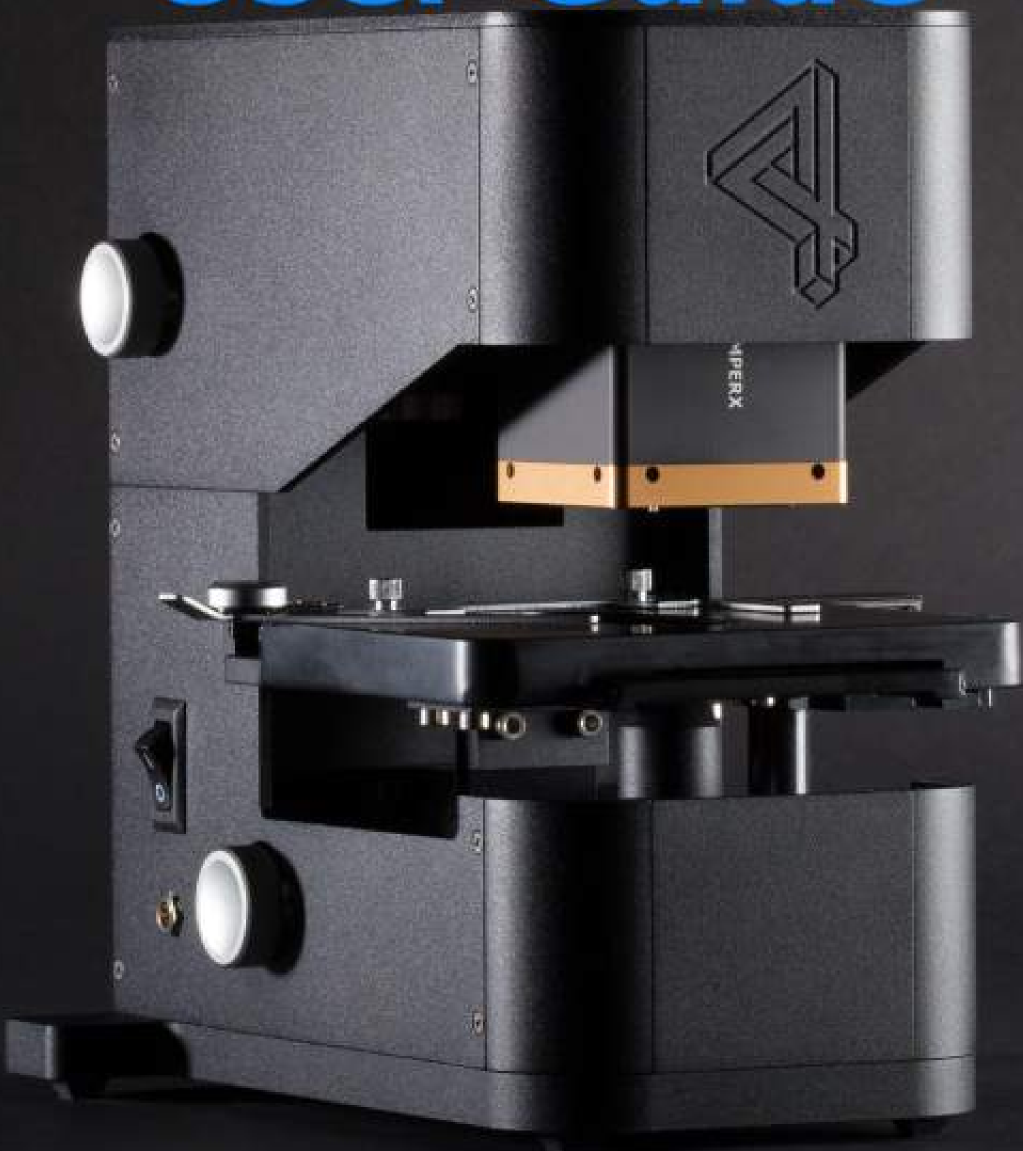
4Deep
inwater imaging

Octopus Software User Guide



Version 1.8.2

Notices

Edition

Octopus User Guide - Version 1.8.2

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Safety and Information Notices

Important

An "Important" signifies helpful information in using the software/hardware. It identifies an important piece of information to guide the user in their workflow, and if not followed could result in time wasted.

Caution

A "Caution" signifies a hazard. It identifies an operating procedure, or step, that if not followed precisely, could result in damage to the product or loss of information. Do not continue beyond a "Caution" sign until the procedure is fully understood.

Warning

A "Warning" signifies a hazard. It identifies an operating procedure, or step, that if not followed precisely, could result in personal injury. Do not continue beyond a "Warning" sign until the procedure is fully understood.

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1 Overview

The Octopus software has been developed to operate in conjunction with any of 4Deep's suite of holographic microscopes (Desktop, Cuvette, Submersible [Standard 500m, 2000m and 2000m+], Autonomous).

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 your 4Deep microscope, please refer to the respective user guide.

The main functions of Octopus are: to *collect* holograms (with a 4Deep microscope), *reconstruct* holograms, and complete *analysis* on individual holograms. For object identification of multiple holograms, see 4Deep's software package Swordfish. For object classification, see 4Deep's software package Stingray.

For example, Octopus can be used 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

1.1 Purpose

Octopus reconstructs and displays 2D images, as well as 3D volumes. The 3D volumes are based on a user-selected number of reconstructed planes within a depth of interest. The resulting images/volumes can be zoomed, rotated and/or panned through, with user-control over levels of transparency, brightness, colour mapping and contouring.

The measurement technique used is digital inline holographic microscopy in transmission. The objects in the holograms, imaged by the microscope, are between 1 um and 2 mm in size.

Octopus can be operated in two modes: live reconstruction, with a reconstruction rate up to 16 fps (2048 x 2048 pixels hologram) or 50 fps (1024 x 1024 pixels hologram), and offline reconstruction (reconstruction of stored holograms), which allows the user to recover all sample information and enables modification of all reconstruction parameters.

1.2 Benefits

Compared to a traditional microscope, holographic microscopy, and the Octopus software have many benefits:

- Accurately measures the thickness of translucent cells and objects.
- Instant: live imaging of samples without any manipulation.
- Simple: no need to focus (can digitally refocus within the volume using Autofocus).

- Fastest performance holograms are reconstructed into images 1000+ times faster than competition.
- Eliminate tasks: capable to count and measure particles automatically in 2D and 3D.
- Examine more interactively: measure and cross-cut objects of interest.
- See more 3D imaging with intensity colour mapping.

2 Installation Guide

2.1 Compatibility

The current version of Octopus (1.8.0) is compatible to run on Microsoft Windows XP SP3 or newer.

Recorded and reconstructed images are exportable in JPG, PNG, BMP formats. Volumes which are created from a hologram are stored as VTK volume files. Particle data from 2D/3D Object Detection is stored in a Microsoft Excel file.

2.2 Installation Package

To install Octopus software on your computer

- Download the software from our website: `http://4-deep.com/software-downloads/`.
- Insert the HASP key supplied and 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.

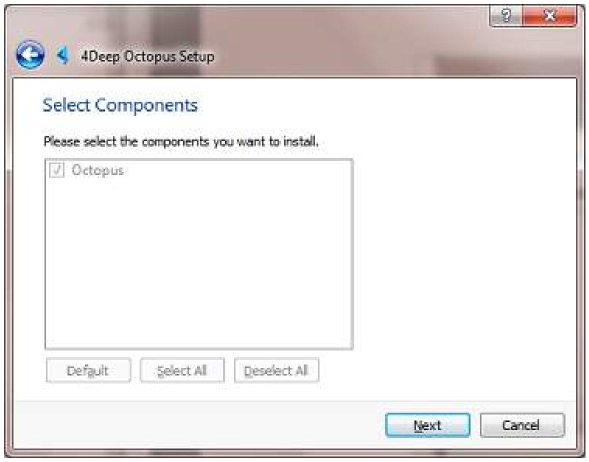


Figure 1: Octopus installer, selection of the installation package

2.3 Connection of the HASP dongle and starting Octopus

After installation, ensure that the supplied HASP hardware protection key (dongle) is connected 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.

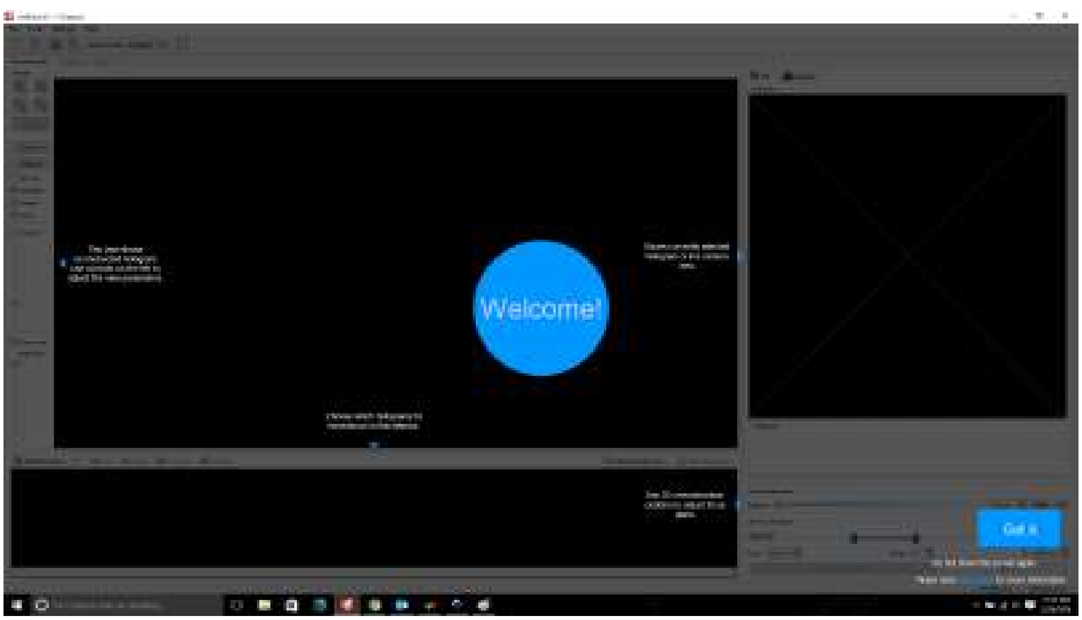


Figure 2: Octopus Software user interface after launch

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. Note that this dialog will only open the first time Octopus is opened, so if you need to change these settings later they are found under Settings -> Reconstruction Options (see Subsection 5.1.1 for more details).

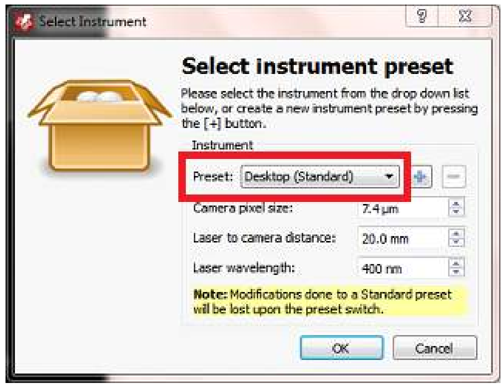


Figure 3: Initial set-up of Octopus based on the microscope used

3 System Overview

This User Guide details the functionality and use of Octopus from a **workflow** perspective. In general, the workflow of Octopus follows four broad categories:

- Collect (Capture or Record) Holograms (Section 4)
- Reconstruct (or View) Holograms (Section 5)
- Analyze Holograms (Section 6)
- Saving (or Exporting) Holograms and Data (Section 7)

For ease of use, the details of each Section listed above will be guided with figures of both the Main Menu (Figure 4) and Main Window (Figure 5), as well as a flow chart of the workflow (Figure 6). The following sections briefly introduce the Home Screen of Octopus to give users a quick overview of its layout.

Important Section 5, Reconstruct Holograms, is where most of the functionality of Octopus lies, and will be where much of your time will be spent.

3.1 Main Menu

Most of the settings and functionality of Octopus is located in the **Main Menu** (see Figure 4). The following gives a brief explanation of each option in the Main Menu (for more details regarding these options, see the Sections referenced).

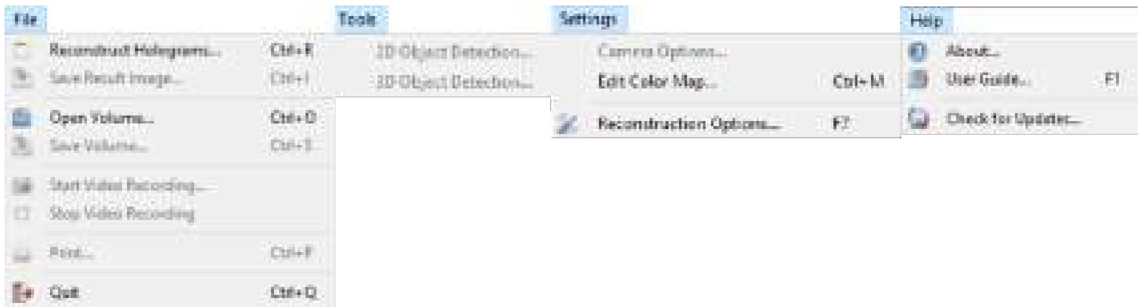


Figure 4: Main Menu, showing the options under each heading

3.1.1 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. You can also select the on the Tool Bar (highlighted in red in Figure 5) to Reconstruct Holograms.

Save Result Image - saves the current view of the reconstructed hologram image to the image file. PNG and BMP formats are supported. You can also select the on the Tool Bar (highlighted in red in Figure 5) to Save Result Image.

Open Volume - opens previously saved reconstructed volume file. VTK volume files and BIORAD PIC files are supported. You can also select the to Open Volume on the Tool Bar (highlighted in red in Figure 5).

Save Volume - saves reconstructed volume to file. VTK volume files are supported. You can also select the to Save a Volume on the Tool Bar (highlighted in red in Figure 5) that has been reconstructed (see Subsection 6.5 for details on Volume Reconstruction).

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.

3.1.2 Tools

2D Object Detection - opens the 2D Object Detection dialog. Refer to the Subsection 6.3 for details.

3D Object Detection - opens the 3D Object Detection dialog. Refer to the Subsection 6.4 for details.

Motorized Stage Controls - opens the Motorized Stage Control. Refer to Subsection 4.1.1 for details.

3.1.3 Settings

Camera Options - opens the Camera Options dialog. Refer to the Subsection 4.3 for details.

Edit Color map - opens the edit color map window. Refer to the dialog description Subsection 5.5.2 for details.

Reconstruction Options - opens the Reconstruction Options dialog. Refer to the Subsection 5.4 for details.

3.1.4 Help

About - shows information about the software.

User Guide - opens software User Guide.

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

3.2 Main Window

The **Main Window** provides users with a view of the holograms they are collecting (smaller, right panel of Figure 5), as well as a view of the reconstructed hologram (larger, left panel of Figure 5). More details regarding the functionality of the Main Window is found later, throughout the text.

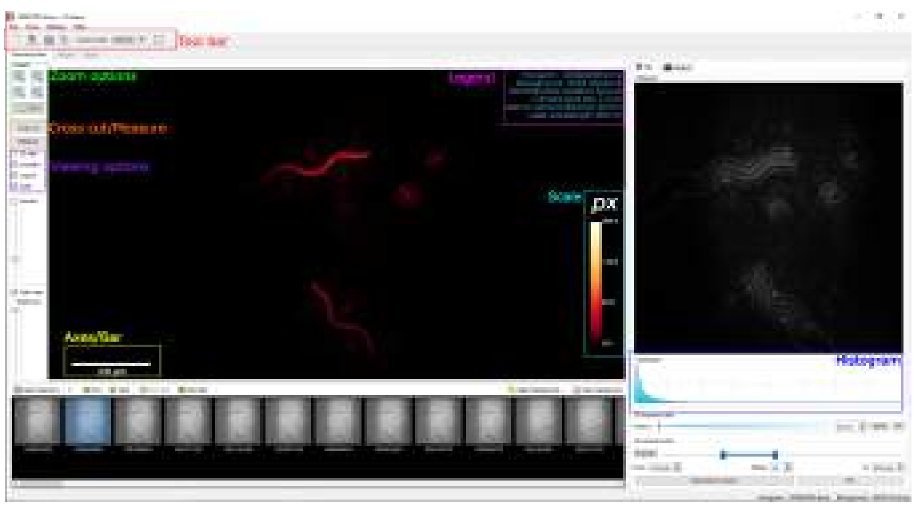


Figure 5: Main Window, with important features highlighted (location of Tool Bar, etc.)

3.3 Work Flow

The general workflow of Octopus follows the four broad categories outlined above (Collect, Reconstruct, Analyze and Save holograms).

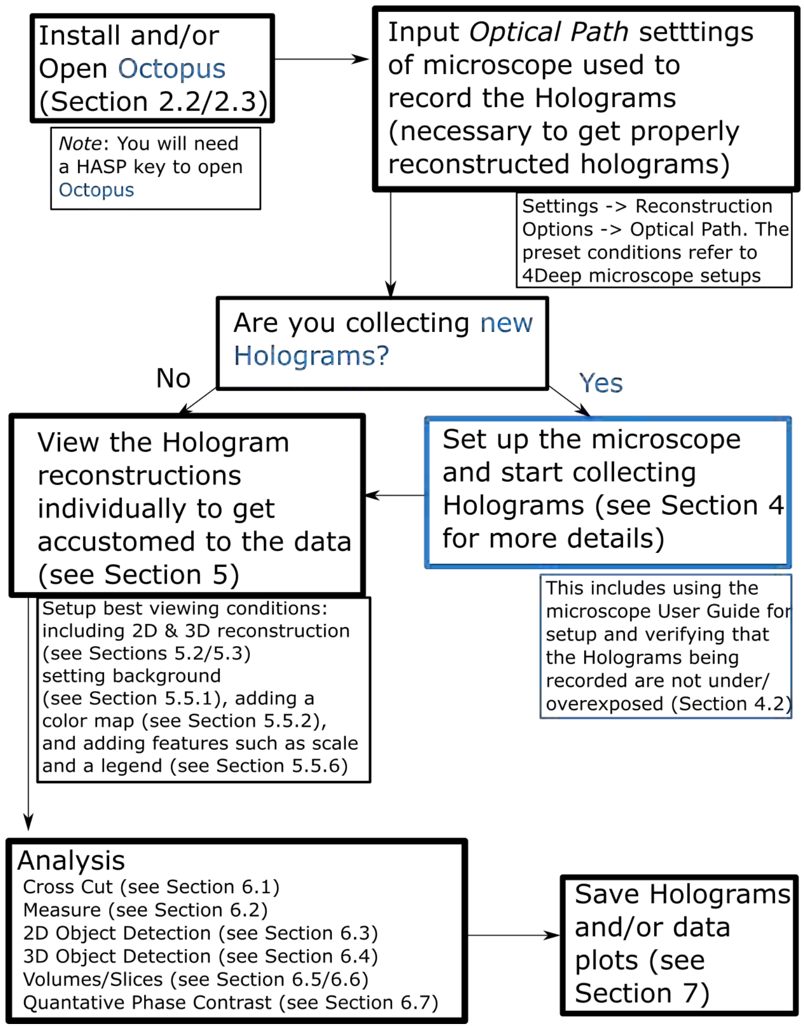


Figure 6: Flow chart of the workflow

4 Collecting the Holograms

Important If you are analyzing already collected holograms, you should go directly to Section 5.

To collect holograms, it is important to setup both the microscope and Octopus properly. The steps below detail how to setup and use Octopus *in conjunction with* a 4Deep microscope. Note that you should be using both this user guide, as well as the user guide for your microscope for complete setup.

4.1 Acquisition setup for Octopus and the microscope

- Set up the microscope and turn it on. Please see the User Guide for the instrument you are using for setup, installation and use before continuing.
- Start Octopus and click on the Camera Tab (right panel in Main Window Figure 5) to connect Octopus to 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. Note: it may take several seconds to connect to the camera. For more help on connecting the camera, see the Troubleshooting Guide (Section 8).

  - The live view from the camera appear 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 (ex: 50 fps for 1024x1024 pixels). All reconstruction parameters can be applied to the real time reconstructions, including the reconstruction position and mode.

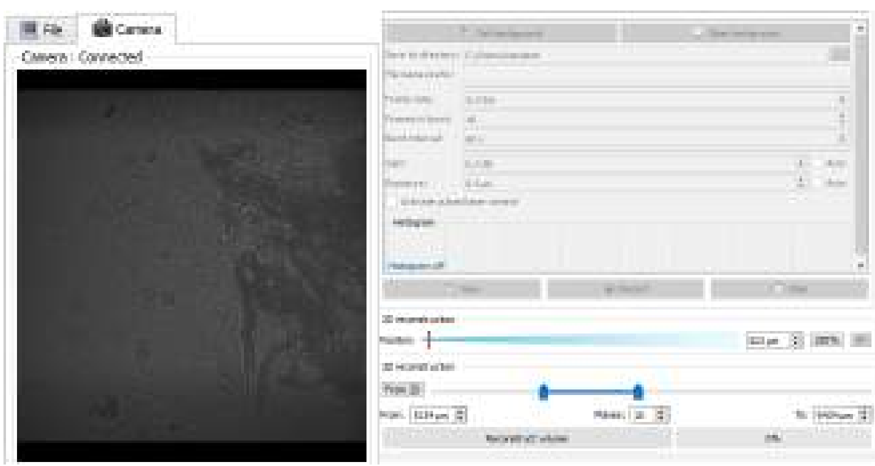


Figure 7: Camera tab view (left panel) and the camera options (right panel)

- Click the "View" button to start viewing (but not recording) the feed
- To properly reconstruct holograms, settings need to be added to Octopus.
  - For the Desktop, select the Preset Option for the Desktop: Settings -> Reconstruction Options-> Preset (see Main Menu Figure 4 for location). If you change the SSD, create a custom preset by pressing the **`Add preset (+)`** button, and give the new preset a name. Any modifications to the preset will be stored when **`OK`** button is pressed. To delete a custom preset, press the **`Remove preset (-)`** button. The pixel size and laser wavelength settings should not need to be changed unless you are using a third-party microscope.

- For other 4Deep microscopes, select the Preset Option for whichever microscope you are using: Settings -> Reconstruction Options-> Preset.
- For third-party microscopes, create a custom preset by pressing the **Add preset (+)** button, and give the new preset a name. Any modifications to the preset will be stored when **OK** button is pressed. To delete a custom preset, press the **Remove preset (-)** button.

Figure 8: Select instrument preset dialog

4.1.1 Motorized Stage Control

Depending on the model of your microscope, it may have a motorized stage which can be controlled with Octopus. A microscope with motorized stage controls will have connector cables from the stage to a power connection on the microscope. If you are unsure if you have a motorized stage, please contact a 4Deep representative....

The open the Motorized Stage Controls,go to Tools -> Motorized Stage Control (see Figure 9). As soon as you click on “Motorized Stage Control”, the sample stage will return to its home position.

You can control the stage by the up/down and left/right arrows on the screen. The step size can be increased or decreased as well. There are also Preset positions (1-6), which can be changed. Set the sample stage at the position you would like to save as a preset, press the “Store preset” button, then select one of 1-6 to store the preset. Using the slider bars, “Position X” and “Position Y”, moves the sample stage to the X, Y coordinate. It will move by the step size, so once you click on the bar, the sample stage will move on its own by the step size until it reaches the position you selected.

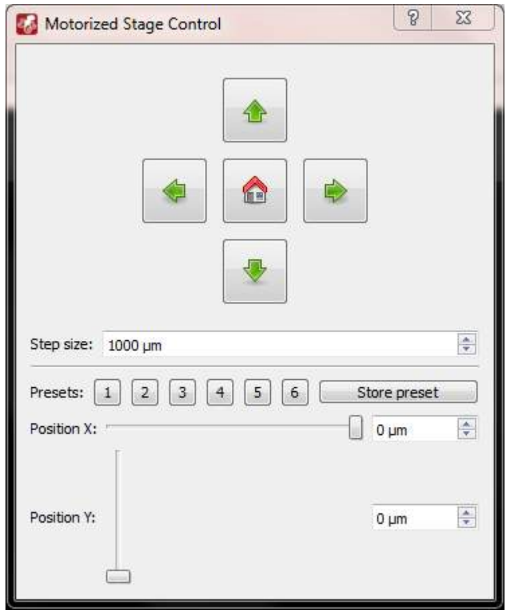
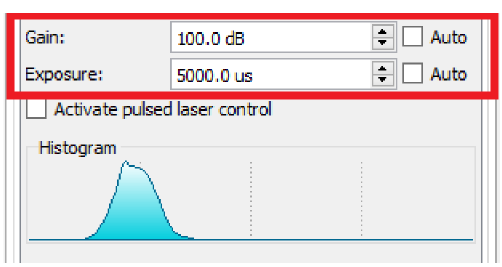


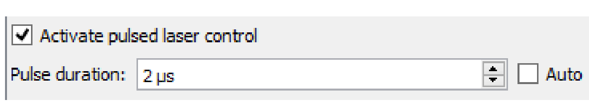
Figure 9: The Motorized Stage Control dialog in Octopus.

4.2 Verifying the exposure quality

Caution Once you have the microscope connected, and Octopus set up for acquisition, it is important to verify that the holograms are not over- or underexposed. Over and under exposure of holograms leads to the loss of information, and subsequent image quality.

Under the camera tab, scroll down until you can see the **histogram** (highlighted in blue in Figure 5, and shown in the figure below). Check the exposure level of the hologram using the histogram. The histogram gives the user a visual cue to the contrast found in the hologram. To correct the exposure quality, 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. Note that if the "Activate pulsed laser control" button is selected, the `Exposure` button will disappear, and the `Pulse duration` button will appear.





Increasing `Gain` artificially increases the intensity of the camera signal. The `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 underexposed, there will be many black pixels, and the **histogram will be to the left**; increase the `Gain` and `Exposure` or `Pulse duration`. These three settings all also have "Auto" tick boxes to automatically select the optimal parameters.

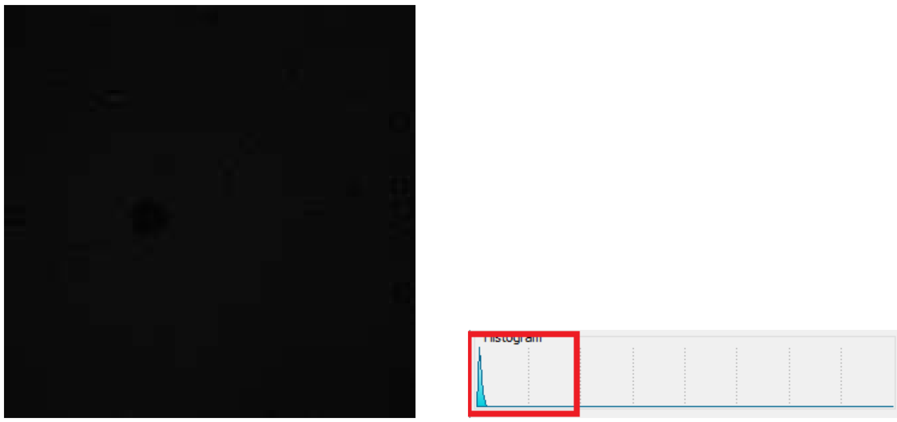


Figure 10: An under exposed hologram, and the histogram associated with under exposure.

If the hologram is overexposed, there will be many white pixels, and the **histogram will be to the right**; decrease the `Gain` and `Exposure` or `Pulse duration`.

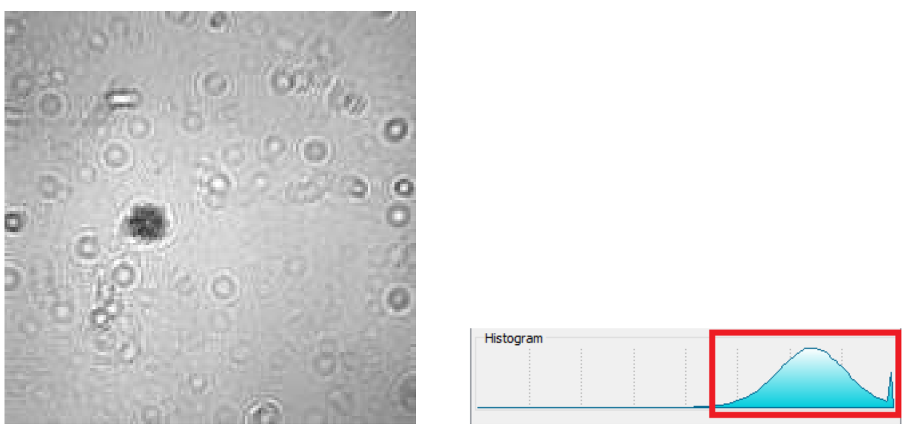


Figure 11: An over exposed hologram, and the histogram associated with over exposure.

The histogram below (Figure 12), with no spikes at the ends, and can be considered a histogram of good holograms. 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.

Figure 12: A properly exposed hologram, and the associated histogram.

4.3 Camera Options and Recording Holograms

At this point, the microscope should be set up, with the correct hologram exposure.

4.3.1 Camera Options

Caution You can change some low level camera options, if necessary. 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.

To open the Camera Options, go to Settings -> Camera Options (note that the Camera Options are only available when a camera is connected). This will open a new dialog box (shown in Figure 13; see Main Menu Figure 4 for the location in the Main Window). The Camera Options dialog provides access to the low level options of the GigE Vision camera installed in the 4Deep microscope.

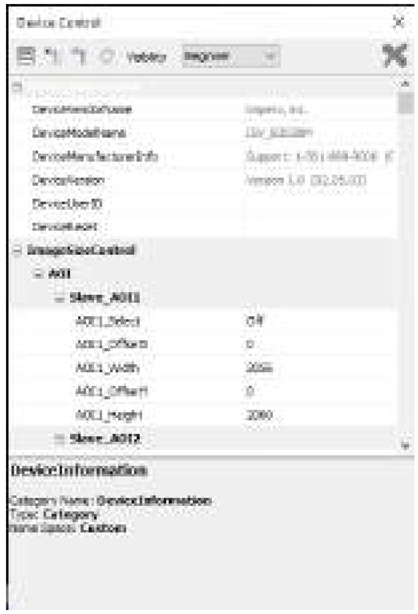


Figure 13: Camera options dialog

4.3.2 Recording options

In the camera tab (Figure 7), there are many settings to change/control.

Camera settings:

When you are viewing or recording holograms, you can use a background, by selecting the "Set background" option in the camera tab. When you select "Set Background", the Octopus software uses the current hologram as a background for the subsequent holograms and presents the background subtraction of the two holograms. You can set a new background by re-clicking "Set background", or clear the background by selecting "Clear background" in the camera tab. Note that the use of a background does not affect the hologram acquisition; the holograms will be saved the same way regardless of the use of a background.

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.

Save to directory - select the directory to which the images will be saved (in BMP format).

Caution Please note that a high capture rate even over a short period of time will take up much storage space on the computer. For example, sampling at a rate of 16 fps for 5 minutes will result in 19.2 GB of data (the size of one frame is 4MB). Please ensure your computer has the space requirements you need before you begin recording so as to not lose important data.

File name prefix - each image file captured by camera will be assigned a name prefix. Each hologram will have the name as a prefix, accompanied by a time stamp.

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. If you set it to be less than 2, it will be the **Continuous run** setting.

Burst interval - interval between bursts (in seconds).

Gain/**Auto Gain** - manual or automatic gain control of the camera. (see Subsection 4.2 for details of use).

Exposure/**Auto Exposure** - manual or automatic exposure control of the camera (see Subsection 4.2 for details of use).

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 (see Subsection 4.2 for details of use). .

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 (see Subsection 4.2 for details).

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 44)

4.3.3 Tips for Acquisition

In order to achieve accurate images, inline holography requires that a reasonable amount of the reference wave (unscattered light) reaches the camera sensor. Too many objects between the point source and the camera can reduce the amount of reference wave reaching the camera and will therefore result in poor reconstructions.

For moving objects, keep the exposure time of the camera as short as possible while still maintaining a visible image. Long exposure times will result in blurry or smeared images. The exposure can be adjusted in the camera control.

The point source does not produce a large amount of light; therefore background light from external sources must be reduced in order to obtain optimal images. Excessive background light can wash out the images and produce low contrast reconstructions.

It is recommended that the user record a test set of holograms initially to ensure all settings are optimized for the objects the user is interested in. It is also highly suggested to obtain a background image (a hologram containing only the light from the point source, without a sample/object causing scatter). A good background image will allow for background subtraction when reconstructing holograms, producing higher contrast reconstructions while reducing noise. A background hologram will also be helpful if there is dust/dirt in the sample area. For more information on using a background, see Subsection 5.5.1.

5 Reconstruct Holograms in Octopus

At this point you should already have holograms collected and saved.

Important The main functionality of Octopus lies in the ability to view Reconstructed holograms (large left panel in the Main Window Figure 5, see Subsection 5.2). This is the aspect of Octopus that you will use the most to view and analyze your holograms. The reconstructed hologram view is different from traditional microscopes because *each* hologram contains information in the Z-plane (the space between the light source and the camera), making the hologram reconstruction 3-dimensional. The objects (ex: oil drops) that you are interested in viewing will come into, and go out of focus as you step though the Z-plane (using the **`Position`** slide) within a hologram. This **`Position`** slide is analogous to the focus knob on a traditional microscope, as it effectively alters the distance between the light source and the camera. Once an object is focused, you can zoom into (see Subsection 5.5.5) and/or save the resulting image of an object (see Section 7).

5.1 Reconstruction Setup and Adding Holograms to Octopus

5.1.1 Reconstruction Setup

To properly scale the holograms, **information about the microscope setup is necessary**, thus the user must input the correct microscope settings. In the Main Menu (see Figure 4), go to Settings -> Reconstruction Options, and find “Optical Path”. Select from the **`presets`** in the drop down menu or input a customized setting.

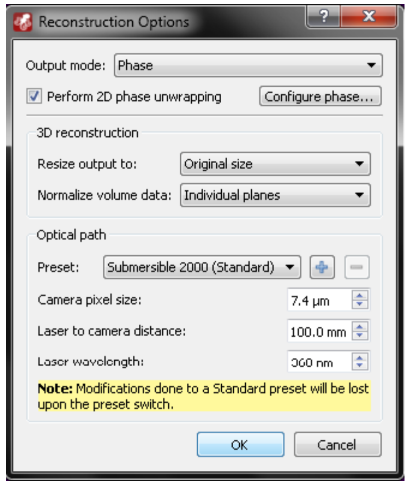
The 4 standard presets: **`Desktop`**, **`Submersible,`** **`Submersible 2k`** and **`Cuvette`** correspond to the optimal configuration of the respective 4Deep microscopes.

Caution 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.

Important If you have just collected holograms (see Section 4), this step should already be completed. Note that if the holograms you are reconstructing were collected with a 4Deep Desktop microscope, you should verify the source to screen distance (SSD) with whoever collected the holograms, as the SSD can be changed by the user.



5.1.2 Adding holograms from a directory

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.

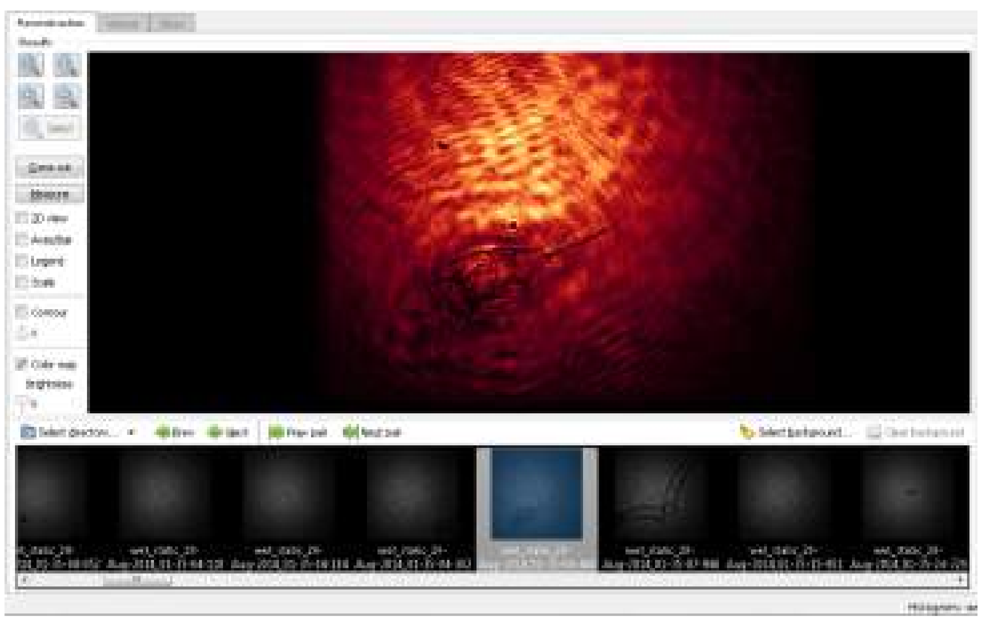
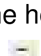


Figure 14: Reconstruction tab, showing the reconstructed hologram (top panel) and the holograms in the directory (bottom panel)

There are mutliple ways to select the hologram(s) to be reconstructed:

- using the Reconstruct Holograms  command (see the Tool Bar in Main Window Figure 5),

- by drag and drop of selected holograms, or

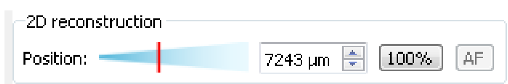
- by clicking **Select directory...** and choosing the holograms from the file preview (see Figure 15).

The resulting holograms will be displayed in the image gallery preview (see Figure 15); 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 (see Figure 15). **Alt+N** and **Alt+P** perform the same function; the **left/right arrow** performs these functions when the image gallery is selected.

Figure 15: The image gallery preview (top) and larger view of control buttons (bottom)

5.2 2D Reconstruction

Once you have added the holograms, you can select holograms one at a time to reconstruct, and view in the main panel in Octopus. The purpose of the 2D reconstruction is to allow the user to step through the hologram to focus on an identify objects of interest.



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.

The **100%** button (to the right of the **Position** slider) 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.

The **AF** button (to the right of the **100%** 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.

Figure 16: Object out of focus

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

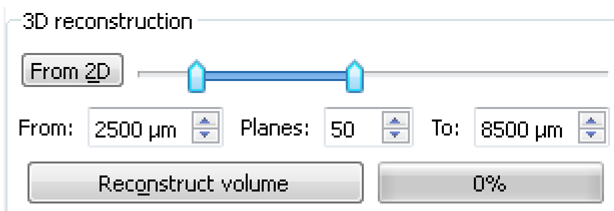
Figure 17: Object from above in focus

NOTE: when reconstructing holograms using a Desktop model, knowledge of the sample depth will be useful. For example, if the user has a 3mm-deep petri dish, and the Source-to-Object distance is 2mm, then all reconstructions should take place when the position slider is 5000 μm or less. Even though the position slider can move past this physical limit, it may prove useful for the user to be aware, especially when manually identifying particles, and at small sizes. This is particularly important at the sub-micron level, as dust particles in the air between the sample and the camera screen will be present around the same scale as objects the user is identifying.

More Reconstruction Options can be found in Subsection 5.4.

5.3 3D Reconstruction

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 (see Subsection 6.5 for more details) and show the reconstructed volume.

More Reconstruction Options can be found in Subsection 5.4.

5.4 Reconstruction Options

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

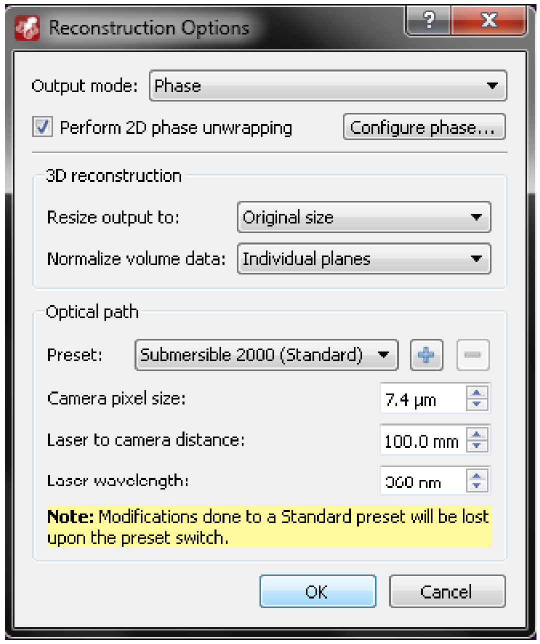


Figure 18: Reconstruction Options dialog

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 (see Subsection 5.1.1 for more details).

5.5 Helpful viewing options

5.5.1 Using a Background

To optimize the examination of a hologram, the use of a background hologram is highly recommended. When a background hologram is used, the user views the difference in the intensities between the two holograms. (NOTE: The background image should be the same size as hologram image for subtraction to work). Selection of a background is usually done one of two ways:

- First, one could use a general background for an entire dataset (eliminates noise and artefacts from the reconstructed holograms). The optimum background to use is a hologram within a dataset that does not have many visible objects. The **`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. The **`Clear background`** button, or **`Alt+L`**, removes the background hologram and reconstructs the hologram with no background subtraction.

- The second way a background proves useful is to view movements/changes from one hologram to the next, by using adjacent pairs of holograms. 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, will be shown in the preview of the loaded hologram.

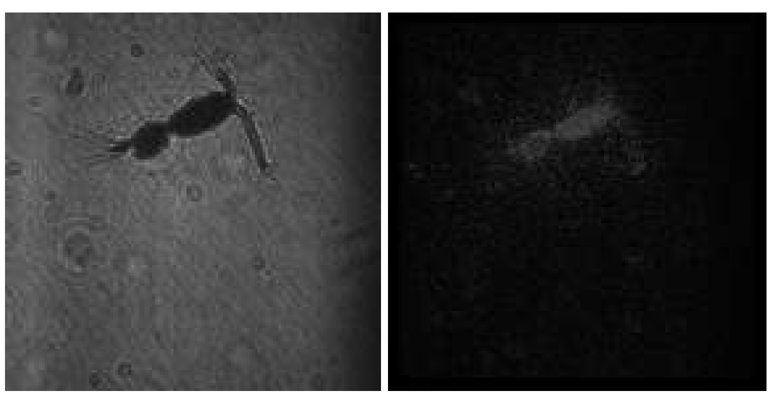


Figure 19: A hologram (left), and the compound hologram (right) after background subtraction

5.5.2 Using a 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 - > Edit Color Map (see Main Menu Figure 4).

A dialog box will appear showing 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. Note that the optimum color map for viewing will be user based.

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.

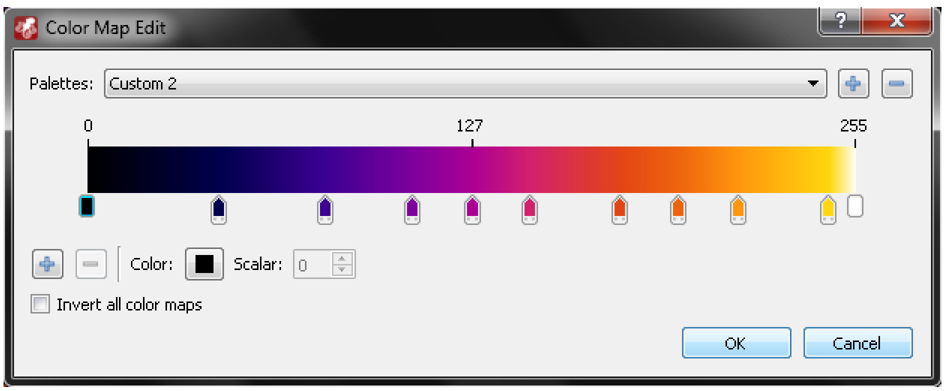


Figure 20: Color map edit dialog

Examples of holograms without a colormap, and with a colormap are shown below:

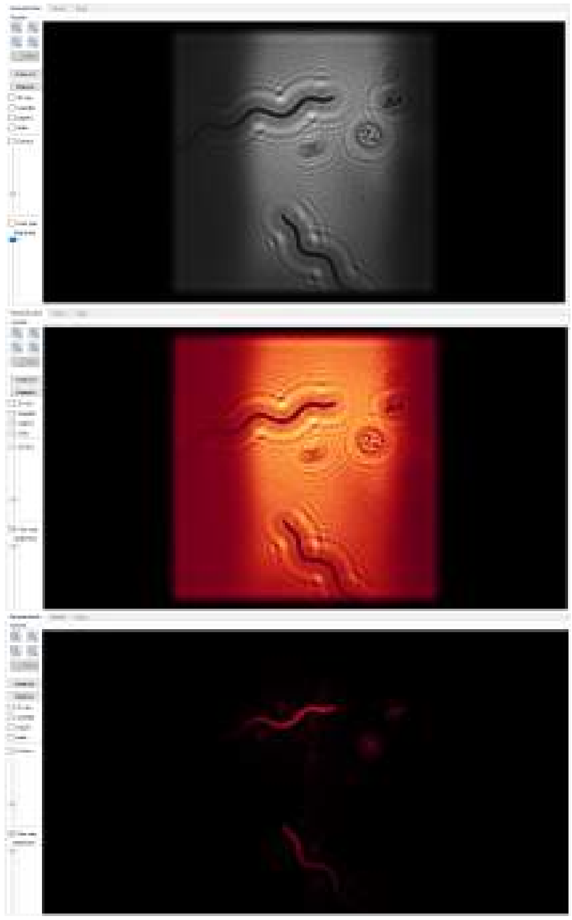


Figure 21: Viewing with and without colormap. The top panel is the raw hologram. The center panel is the view of a hologram with the colormap called "sequential orange". The bottom panel shows a hologram with both a colormap and background subtraction.

5.5.3 3D view

Checking the 3D view box (highlighted in purple as "Viewing Options" in the Main Window Figure 5) 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.

5.5.4 Full screen

Full screen - Opens the 2D reconstruction window (or the 3D view) to the full screen, `F11` works as a hot key (highlighted in red in Figure 5) . 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.

5.5.5 Zooming options

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.

The zooming options are highlighted in green in the Main Window Figure 5. Once you have zoomed into an object in the hologram, you can save the resulting image (see Section 7).

If you have zoomed into an object within a hologram, you can pan through the full hologram using “Shift” on the keyboard + the mouse to point-and-click-and-drag.

5.5.6 Legend, Axes/Bar and Scale

Checking the boxes (highlighted in purple as “Viewing Options” in the Main Window Figure 5) will produce the legend on the upper right of the reconstructed image (highlighted in pink in the Main Window Figure 5), the axes along the lower and left hand side of the image (highlighted in yellow in the Main Window Figure 5), with a second click giving the calibration bar at the lower left, and the intensity scale in the bottom right corner (highlighted in cyan in the Main Window Figure 5) . Legend, axes, bar and scale are saved on the result images.

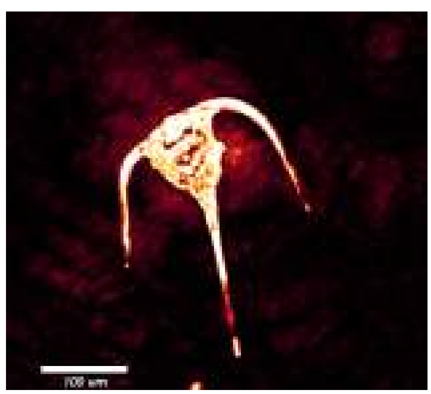


Figure 22: Calibrated Bar for reconstructed images

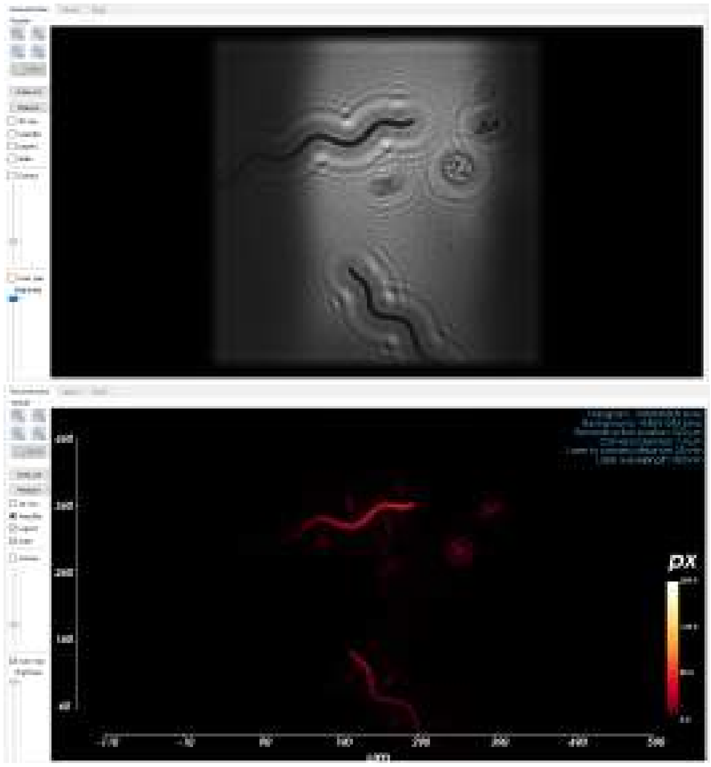


Figure 23: A reconstructed hologram without (top) and with (both) legend, axes and scale.

5.5.7 Contour

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

Figure 24: Contour selection tool

6 Analyzing Holograms

At this point, you should have holograms saved and added into Octopus, with settings such that you can see the holograms clearly. Using the 2D and/or 3D Reconstruction you should now be comfortable with your dataset, recognize objects in your dataset, and have an idea about what analyzes you will wish to complete.

6.1 Cross Cut

The **Cross cut** button (highlighted in orange in the Main Window Figure 5) 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.

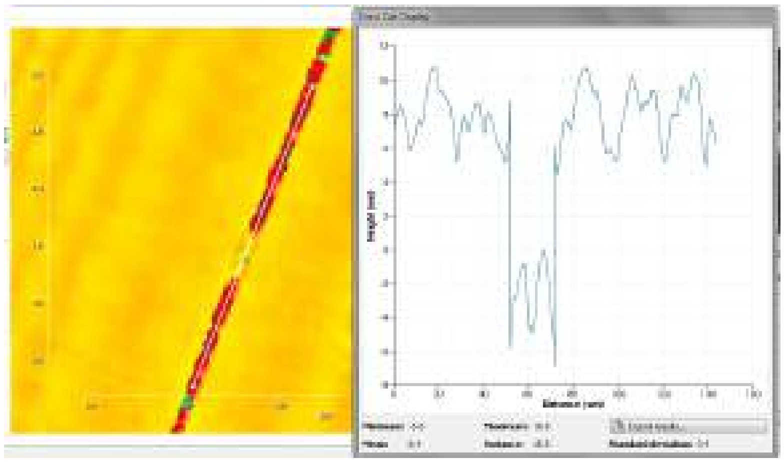


Figure 25: Crosscut function

6.2 Measure

With the **Measure** button (highlighted in orange in the Main Window Figure 5), 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.

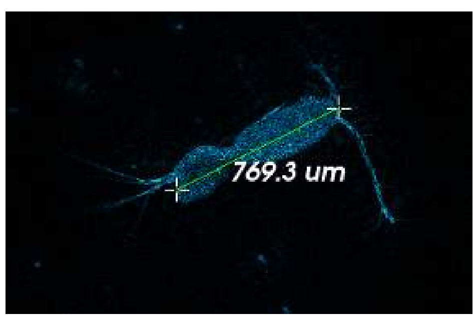


Figure 26: Measuring a reconstructed image

6.3 2D Object Detection

To detect objects in 2D, go to Tools -> 2D Object Detection (see Main Menu Figure 4).

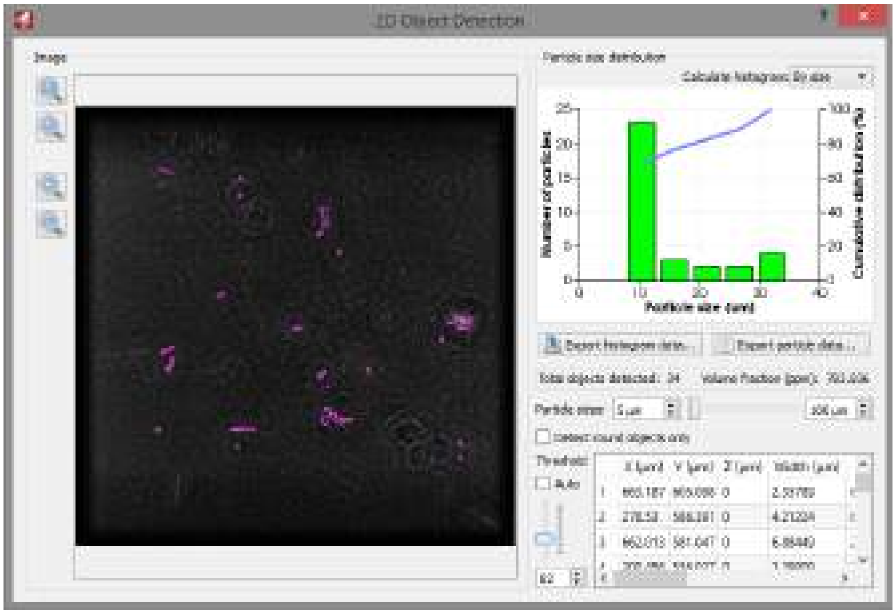


Figure 27: 2D object detection dialog

This will open up the 2D object detection dialog, which 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 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`, `Volume`, or `D[4,3]` option is calculated in each histogram bin). More details on this can be found in the Swordfish User Guide.

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.

6.3.1 An example of 2D Object Detection

The following is a simple example of how to use 2D Object Detection. In particular, it shows how it is useful, and how and where to use the settings:

When you use the 2D Object Detection, the two main settings used to detect objects are the threshold and the size range.

- Use a general background (see Subsection 5.5.1) to remove noise from the environment
- In the Reconstruction Tab, focus on the object you are interested in (using the hot keys W+X and/or A+D)
- In the Reconstruction Tab, use the Measure Tool (Subsection 6.2) to measure the length and width of the object of interest

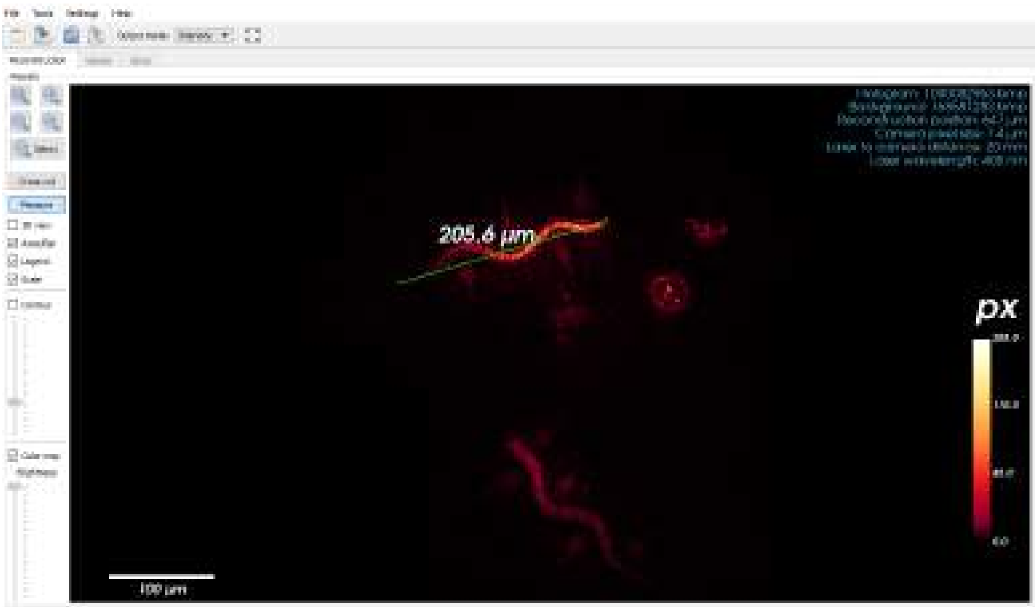


Figure 28: Measuring the object of interest

- Use these settings as limits in the particle size range (Tools -> 2D Object Detection). For example, the object above was measured about 200 µm by 17 µm. Thus, set the size range in the 2D Object Detection as 15 to 210 µm (in the red box in Figure 29).

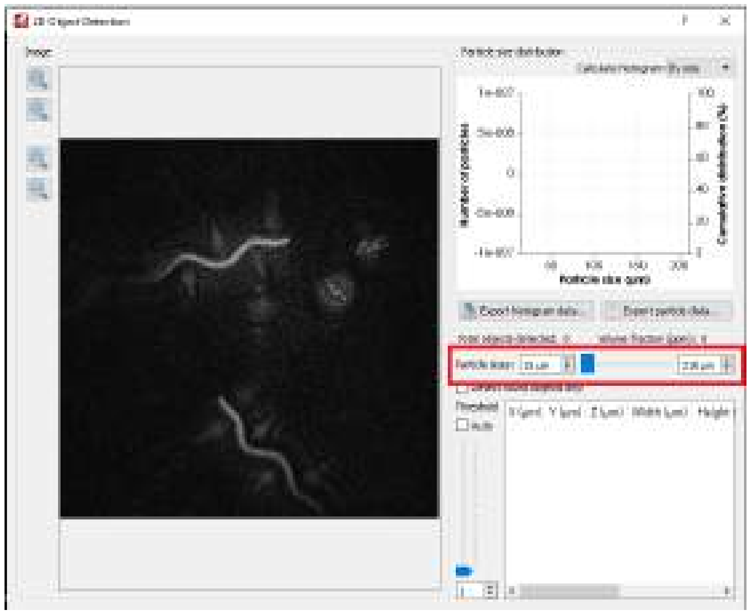


Figure 29: Setting the size range for object detection

- Once the size range is set, the threshold can be changed. A high threshold, for example 200, means that the intensity of the pixel has to be 200 or more for that pixel to be detected by the software. Thus, a low threshold (ex: 5) would allow many pixels to be detected, while a high threshold (ex: 200), would let less pixels be detected. Start with a low threshold and work your way up to a higher threshold. There is also an “Auto” option for threshold.

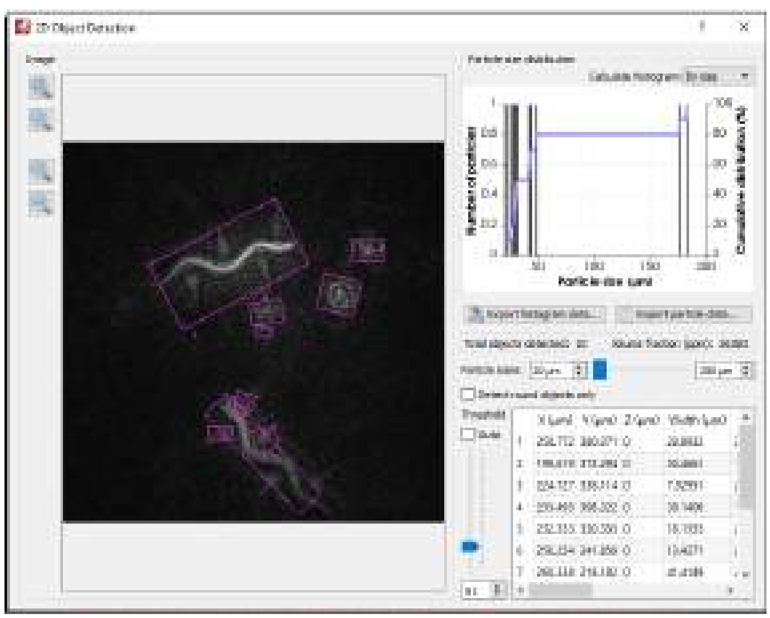


Figure 30: Threshold for object detection

- Finally, there may be a need to tweak each setting until all of the objects of interest are detected by the settings

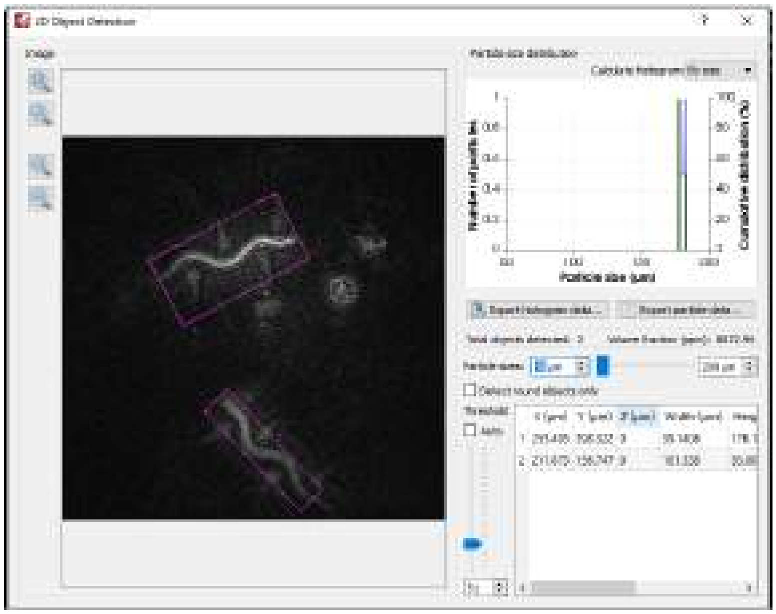


Figure 31: Final tweaking of the settings to detect the objects of interest

To detect objects from more than one hologram at a time, please see Swordfish.

Important If you are using Object Detection (2D or 3D) in Swordfish for a dataset, it is recommended that you use Octopus to identify settings which work for the identification. For example, if you are analyzing oil drops, in Octopus, you will want to set the minimum and maximum particle size, as well as an intensity Threshold. Then using the display window in the 2D Object Detection, you can see (magenta boxes) whether an object will be identified with these settings. Once the settings in Octopus give you the results you want, you can use the same settings in Swordfish to identify/analyze many holograms at once in Swordfish. The process described in the example above may need to be completed with multiple holograms in the dataset to ensure that the settings allow for detection of all objects of interest.

6.4 3D Object Detection

To detect objects in 3D, go to Tools -> 3D Object Detection (see Main Menu Figure 4).

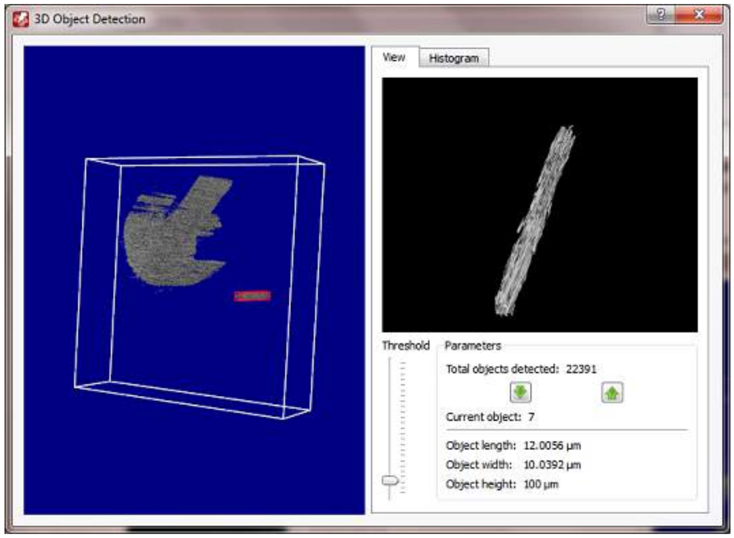


Figure 32: 3D object detection dialog

This opens up the 3D Object Detection, which 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.

6.5 Volume Tab

After 3D reconstruction (see Subsection 5.3) , the reconstructed volume is shown in the Volume Tab.

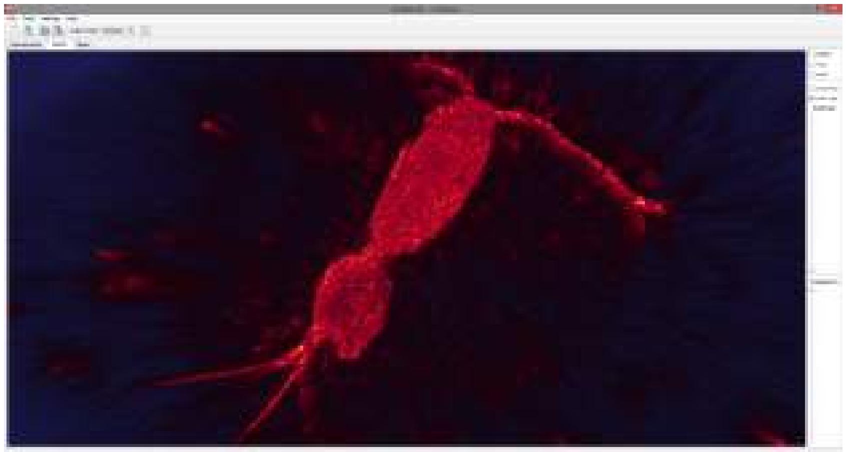


Figure 33: Volume reconstruction

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.

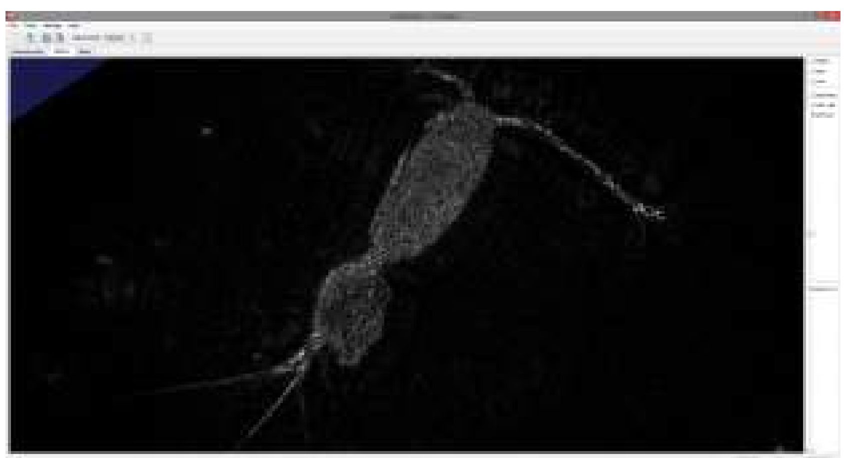


Figure 34: Volume view, grayscale mode, 0% transparency

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 35: 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.

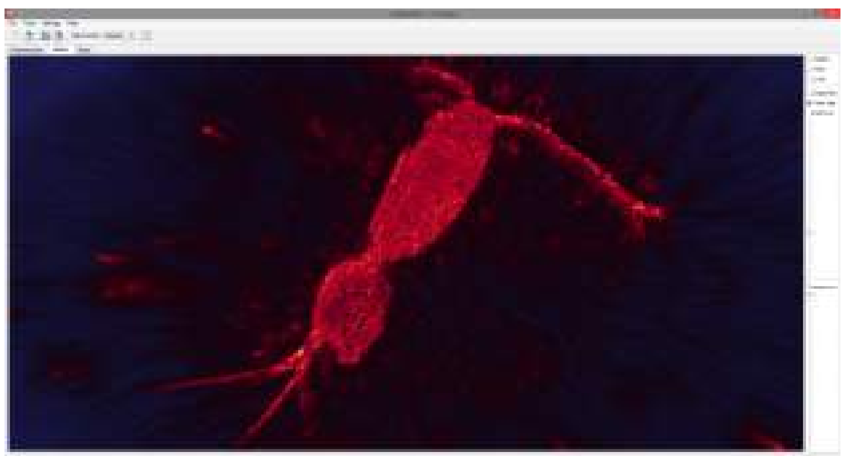


Figure 36: Volume view, colormap mode, 100% transparency

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 37: 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.

6.6 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.

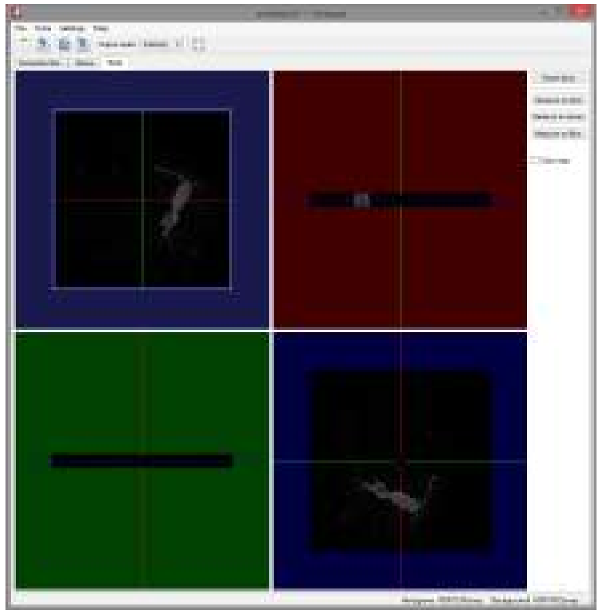


Figure 38: View of slices tab

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.

6.7 Quantitative Phase Contrast Imaging

To begin Quantitative Phase Contrast Imaging, go to Settings -> Reconstruction Options (see Main Menu Figure 4) and select `phase` output from the drop down menu.

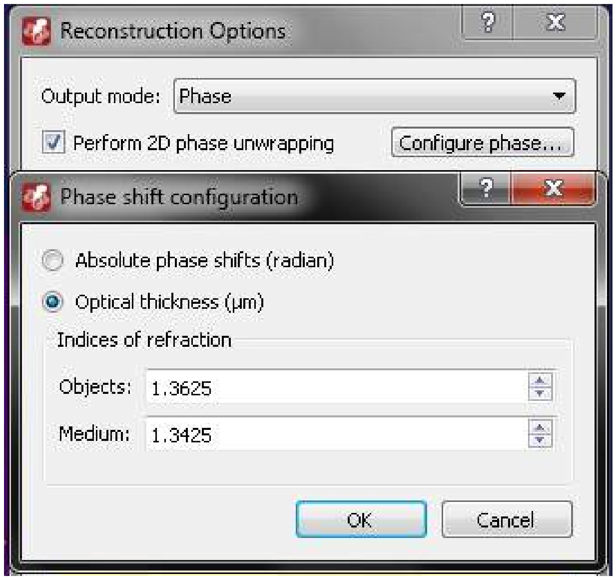


Figure 39: Phase shift configuration dialog

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.

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.

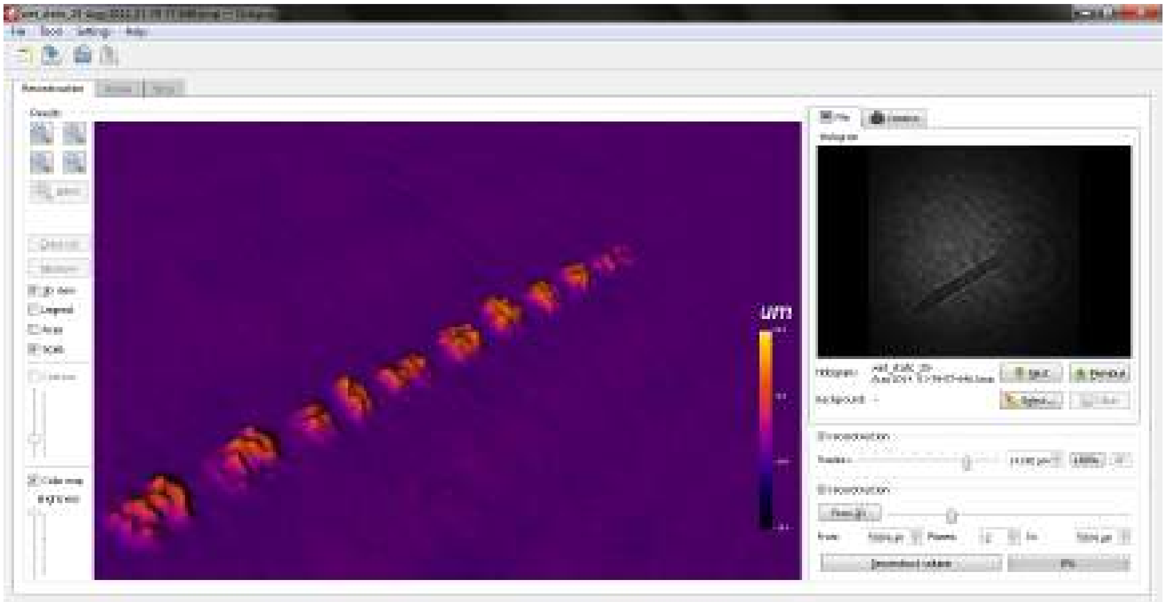


Figure 40: 3D view of quantitative phase contrast imaging in Octopus

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`

if you are using a color map, and switched from viewing the Intensity to viewing the Phase, you will notice the color map values are now in microns from the reconstruction plane, giving the optical thickness of the object. With the **`Scale`** selected in this output, the colours indicate thickness, with the scale corresponding to the thickness of the object in microns.

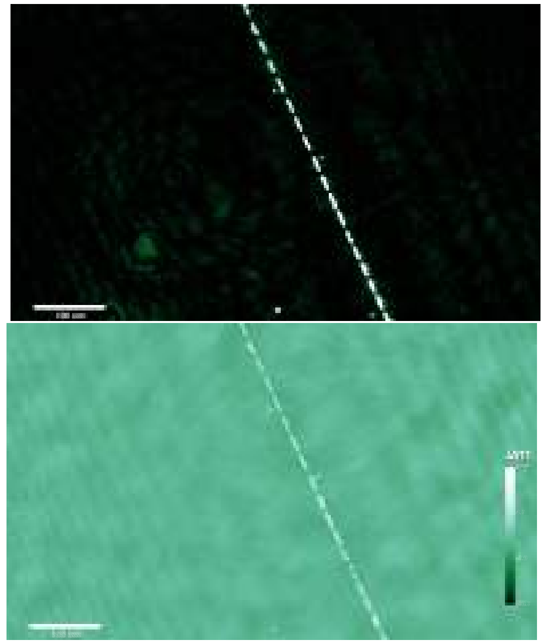


Figure 41: Reconstruction of an algae chain using intensity and background subtraction (left), and the same hologram represented in phase information; the scale on the right reads out in microns.

An optimal way of using phase reconstructions is with the **`Cross cut`** tool (highlighted in orange in the Main Window Figure 5) . 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.

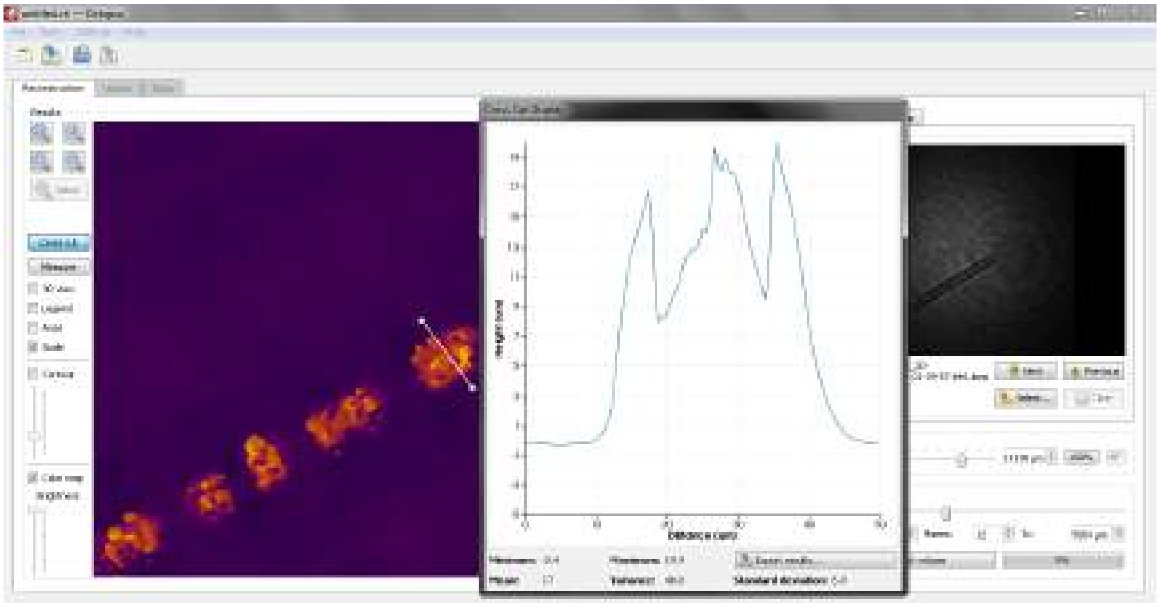


Figure 42: Crosscut of quantitative phase contrast imaging in Octopus

6.8 Creating a Video Replay of the Holograms

One interesting application of Octopus is the ability to record videos of already recorded holograms. This is beneficial to create a replay of hologram(s) both through time and the Z-axis. The video option can record each step from one hologram to the next, as well as step through the Z-axis within one hologram. The controls to start and stop recording are located under “File” (see Figure 4).

7 Saving and Printing Holograms and Resulting Images

At this point, you have recorded, reconstructed and analyzed holograms.

There are several results from Octopus you can save if required, outlined in the Table below:

Type of Result	How to Save
Resulting Image	If you have zoomed into an object in a hologram, and want to save that frame, you can do so by File-> Save Resulting Image (see Main Menu Figure 4) as a *.jpg, *.png or *.bmp. The default directory is the last directory where something was saved. Important It is helpful to save an image with the Legend (see Subsection 5.5.6) on, as it contains important information regarding the image, including the hologram name, the background hologram and the distance from the camera (all pertinent information to re-create the image).
Cross cut results	Save from the Cross cut window, using “Export results” and save as a Microsoft Excel spreadsheet
Data from Object Detection	Save from the Object Detection Window. You can either “Export histogram data” or “Export particle data” (or both) as a Microsoft Excel spreadsheet
3D Volume Reconstruction	Once a 3D Volume is constructed, it can be saved by File -> Save Volume (VTK file)
Video replay of Holograms	You can save a replay of stepping through holograms by going to File -> Start Video Recording and stop recording by File -> Stop Video Recording

You can also Print an image from a hologram by going to File -> Print (see Main Menu Figure 4). Again, if you have zoomed into an object, the image printed will be the zoomed-in view of the object, as with saving.

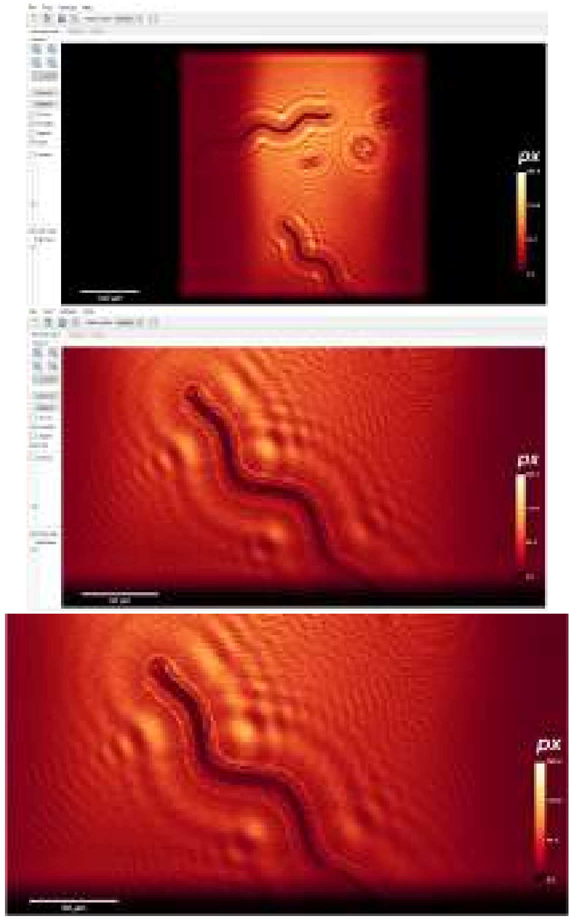


Figure 43: Normal (no zoom) view (top) and zoomed-in view (center) of an object in a hologram, and the resulting image if printed or saved (bottom).

8 Troubleshooting

The most common issues, with Troubleshooting solutions listed below.

Issue	Solution
Camera not connecting in Octopus	Check that the cables are properly attached, and power is on If there are more than one Ethernet ports on the computer, try a different one If you are using a laptop computer docking station, use the Ether net port on the docking station (not the laptop itself)
Holograms are over/underexposed	Adjust Gain, Exposure or Pulse duration (see Subsection 4.2 for more details). For moving objects, keep exposure time as short as possible because long exposure times will result in blurry images
Unable to locate Objects in hologram	There may not be enough light to develop a proper reference wave. Ensure your sample is aqueous/transparent There may be too many objects between the point source and camera to produce a proper reference wave. Try pre-filtering or using a smaller sample volume. Use a background hologram to reduce background noise. (see Subsection 5.5.1)
Unable to focus on Objects in hologram	The Source-to-Screen Distance (SSD) may not be properly defined in the Reconstruction Options. Measure the SSD and ensure it is changed in the Reconstruction Options. See Subsection 5.1.1 for further detail
Point source not producing much light	Background light must be reduced to obtain optimal images. Try using a light shield (standard with Desktop microscope)
HASP key is not detected	Ensure the HASP key is plugged in. Try a different USB port. Download the HASP driver from www.4-deep.com under Products -> Software Downloads

Table 1: Common troubleshooting problems and solutions

9 Appendix

9.1 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

Table 2: Hot Key Guide

9.2 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 you 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 μs, 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 μs.

`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.

`RECONSTRUCT_HOLOGRAMS Val\n`

Selects which holograms to reconstruct. Value is a list of hologram file names, separated by * character. You can either use absolute paths, or paths relative to the currently selected hologram directory. If more than 1 file is contained in the list, background subtraction will be applied.

`SAVE_RESULT_IMAGE Val\n`

Saves reconstructed image to file. Value is the absolute path to image. Image file extension determines the type of image file (PNG, JPEG, TIFF, BMP) that is being saved.

`RECONSTRUCTION_POSITION Val\n`

Reconstruction position (in μm) for 2D reconstruction.

`OUTPUT_MODE Val\n`

Sets output mode for reconstructions. Value = 0 intensity reconstructions; 1 amplitude reconstructions; 2 phase reconstructions.

`DISTANCE_FROM Val\n`

Distance from reconstruction position (in μm) for 3D reconstruction.

`DISTANCE_TO Val\n`

Distance to reconstruction position (in μm) for 3D reconstruction.

`PLANES_COUNT Val\n`

Number of planes to reconstruct for 3D reconstruction.

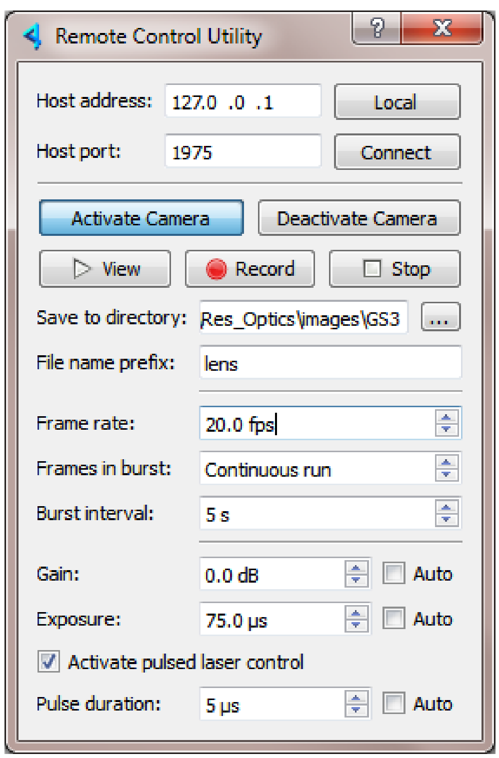


Figure 44: Camera Remote Control Utility

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.

9.3 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 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.

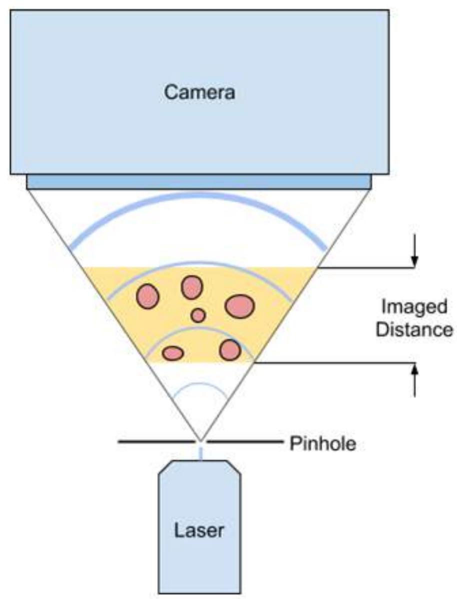


Figure 45: The basic principle of digital in-line holographic imaging

9.4 The Advantages of Holographic Microscopy

The images obtained from holographic microscopy, such as Figure 12, 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.

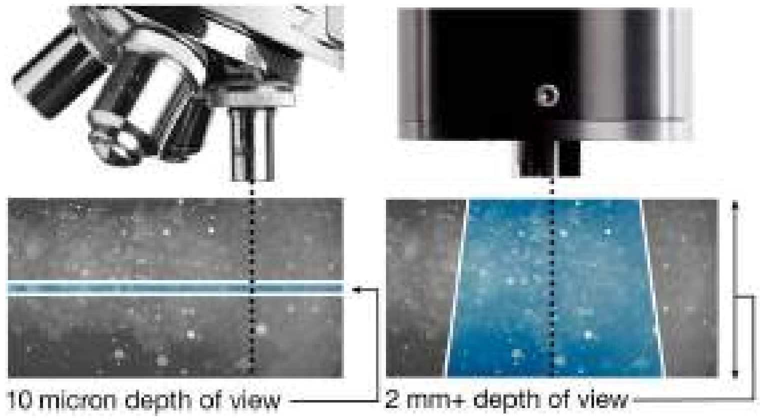


Figure 46: Field of view comparison of lens based microscope (left) and holographic microscope (right)

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.

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