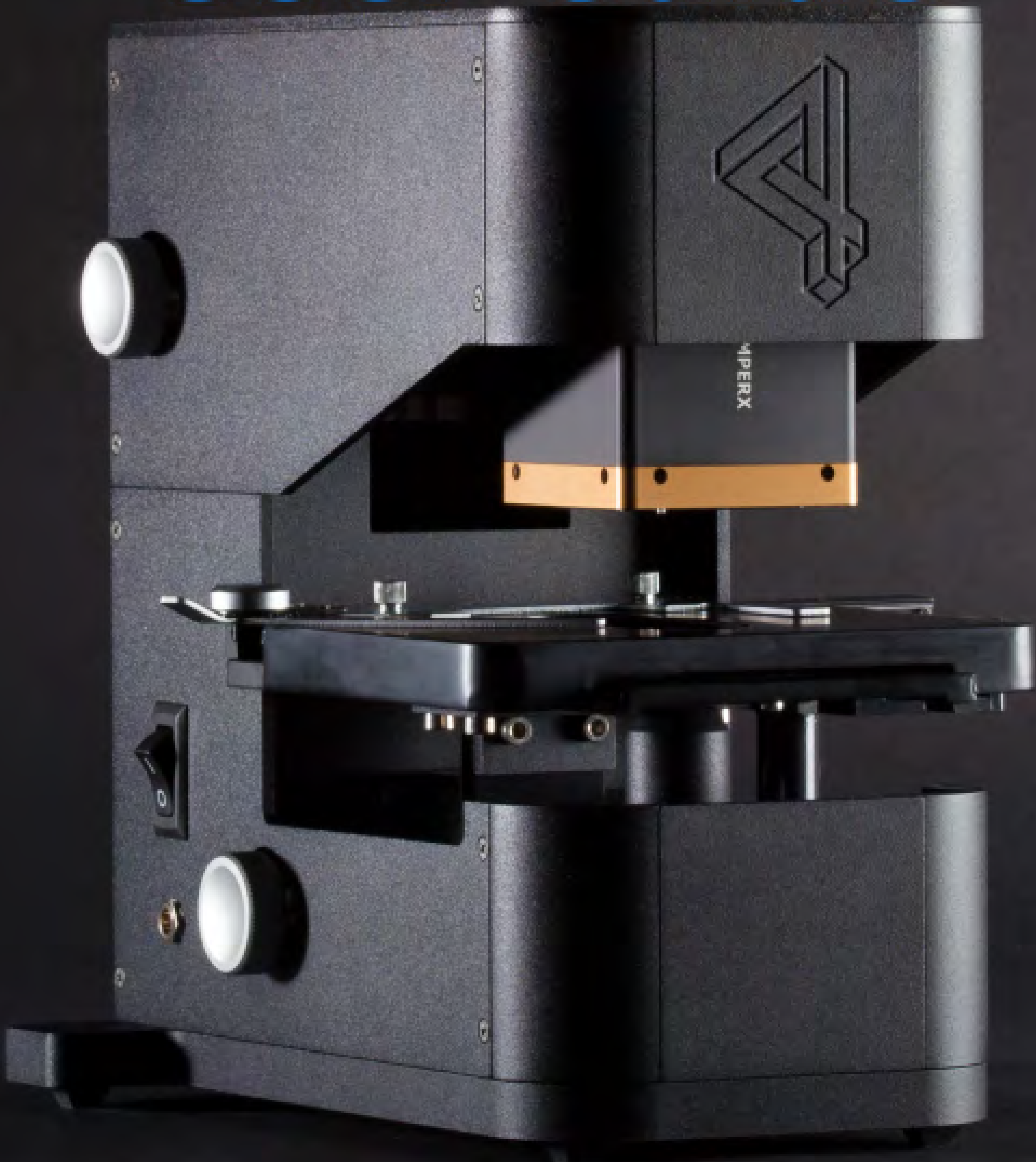
4Deep
inwater imaging

Desktop Microscope User Guide



Version 1.1.0

Notices

Edition

Desktop Holographic Microscope User Guide - Version 1.1.0

4Deep inwater imaging

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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.

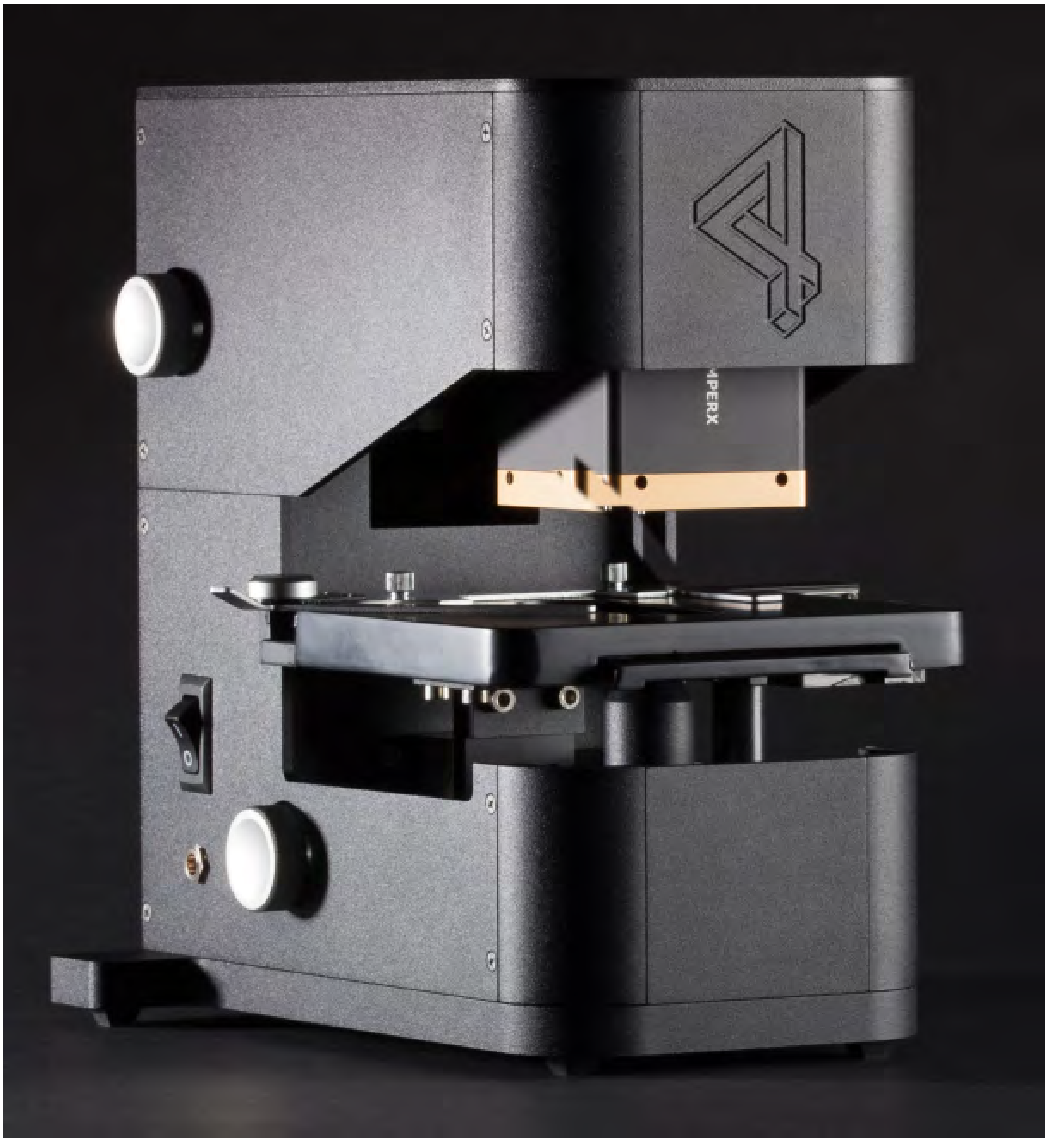
Caution

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.

Desktop Microscope Version 1.1.0



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1 Overview

The Desktop Microscope has been developed to operate in conjunction with 4Deep's Octopus (and/or Swordfish) software. Note that hologram collection cannot be completed without the use of 4Deep software.

This user guide is strictly related to the installation, use, care of and functionality of the Desktop microscope. For a detailed description of the setup and operation of the 4Deep software, please refer to the respective user guides.

The Desktop Microscope is a benchtop microscope; for the in-water microscope, see the Submersible Microscope (0-500m, 2000m or 2000+ m).

The Desktop Microscope can be used to collect images from the fields of:

- 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

The Desktop microscope comes from a suite of instruments that all operate under proprietary digital inline holography technology platform, developed by 4Deep. With its new and unique method of data acquisition and analysis, this system is designed to optimize your research of the microscopic world. The microscope is able to capture dynamic movement and is useful for time-dependent experiments. The Desktop microscope is ideal for tracking moving samples through the depth of view (Z-plane), and for transparent and translucent fluid suspensions or dry samples up to several mm.

The measurement technique used is digital inline holographic microscopy in transmission. The light source is a single source of 405 nm. The sample stage has a manual 2 axis x-y travel with 30 mm of travel available.

1.2 Benefits

Compared to a traditional microscope, the holographic microscope has many benefits:

- HD quality in 2D, 3D and 4D representations.
- Portable: can be used in the lab or in the field.
- Instant: live imaging of samples without any manipulation.
- Effortless: no staining or prep work required.
- Compact: minimal footprint in laboratory settings.
- Simple: no need to focus.

2 Introduction

2.1 Safety Information

The user should read this user guide and any other additional information supplied by 4Deep before operating the instrument.

Warning This microscope contains a class IIIB Laser. Up to 100 mW at 405 nm is emitted from the laser housing itself. If properly assembled, the light emitted from the point source should not pose any threat of long term damage to an exposed eye. **There are no user serviceable parts inside the light source**. If problems are encountered regarding the light source, **please return it immediately for service**. Allow only a qualified technician to disassemble the point source. ***Do not attempt to remedy any problems with the light source on your own!***

Caution Always make sure that the proper 12 VDC power supply is used with the microscope. Failure to do so could result in critical damage to the microscope.

2.2 Compatibility

As mentioned above, the Desktop microscope needs to be used in conjunction with the Octopus software. The computer requirements are: a PC with Intel Core 5 and a Nvidia GTX or better graphics accelerator card. The data is transferred via a Gigabit Ethernet port to the host computer.

2.3 Components of the System

Important Be careful when unpacking the contents of the package. As the 4Deep microscope images at the micron/sub-micron level, foreign objects (dust, dirt, fingerprints, smudges, perspiration, saliva etc.) on the camera lens, the point source and/or the sample holder can greatly affect the quality of the captured holograms. Take care not to touch the camera lens, the point source and both side of the glass in the sample holder. For further Care and Cleaning information, see Section 4.

The following is the list of parts of the Desktop microscope.

- Desktop microscope, pre-assembled. For full details, see the microscope description in Section 3.3.

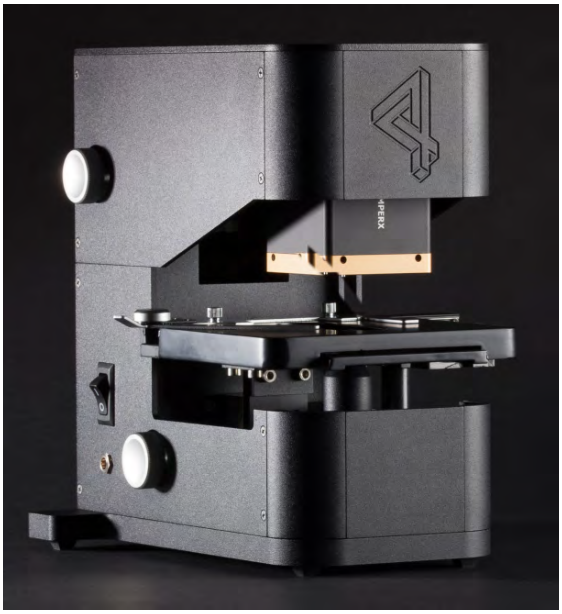


Figure 1: Pre-assembled Desktop microscope

- Power supply (12 VDC).

Figure 2: Power supply

- Ethernet cable.

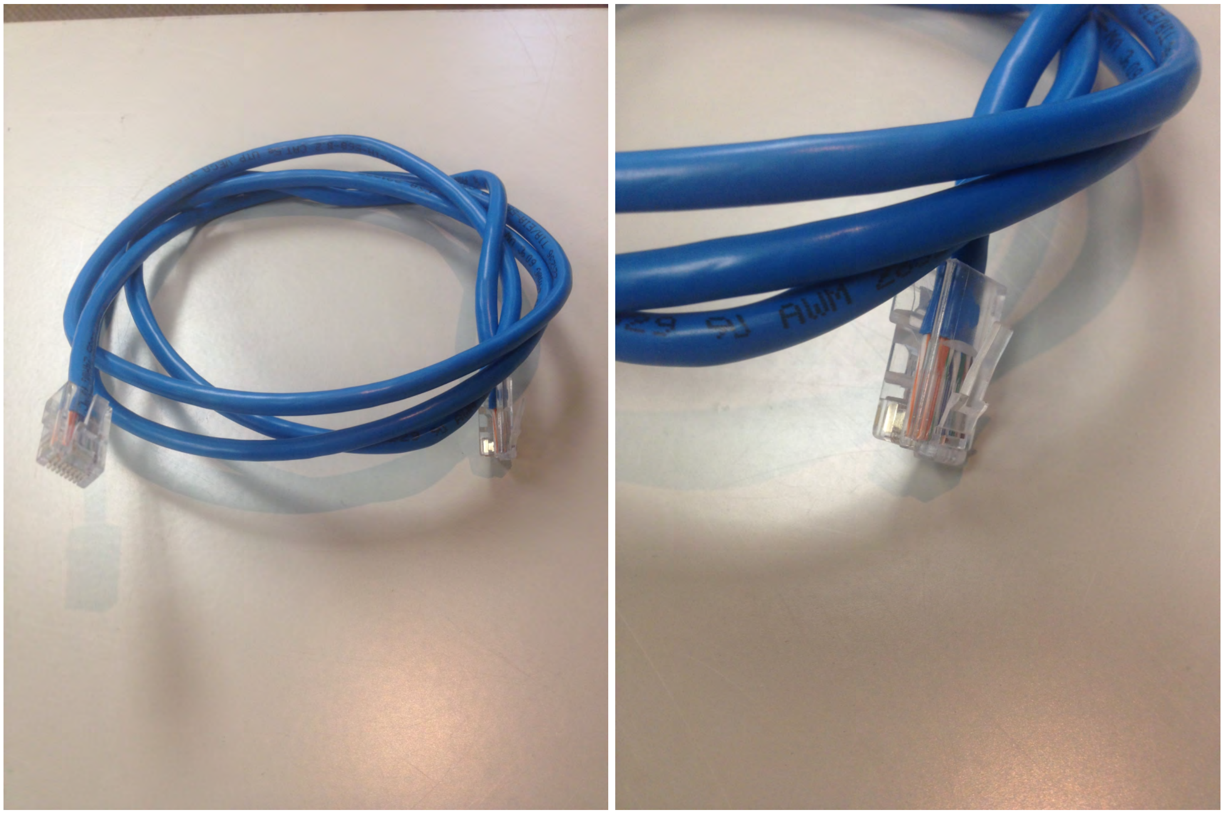


Figure 3: Ethernet cable from a distance (left panel) and close-up (right panel) of the connector

- Sample holder.

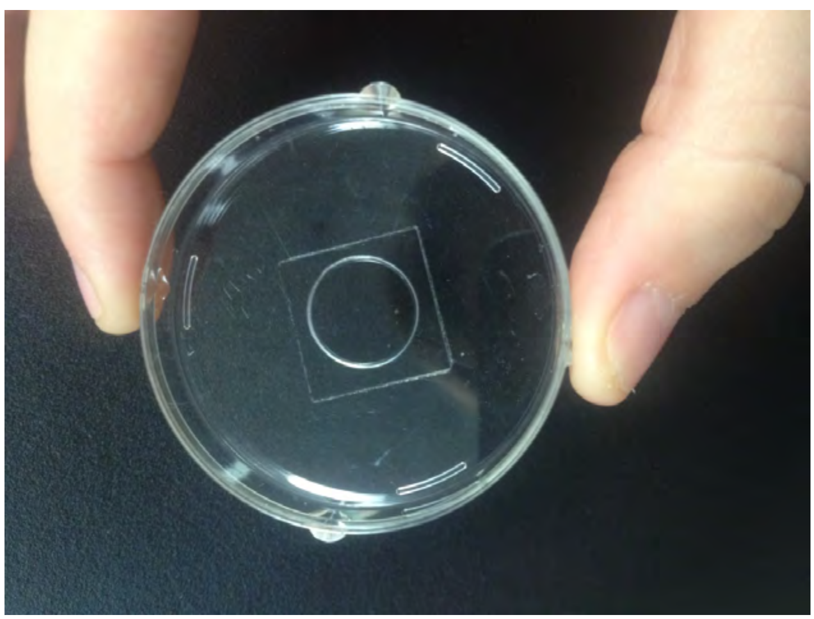


Figure 4: Sample holder (small petri dish with hole for slide glass)

- Light -shield

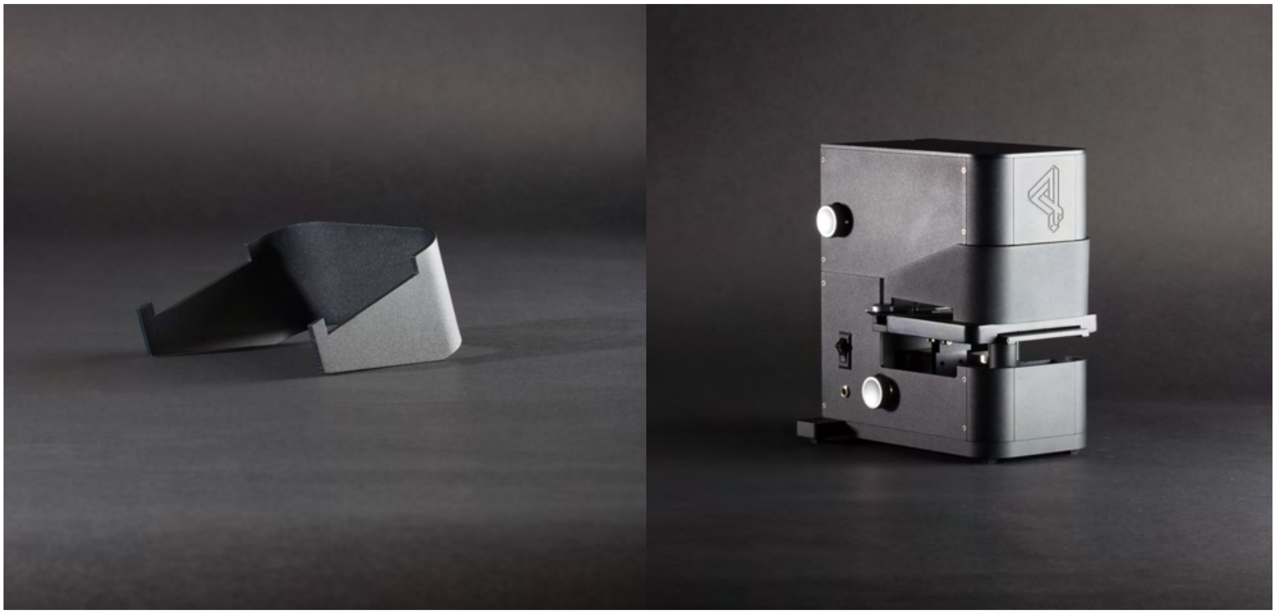


Figure 5: Desktop shield (left panel) and Desktop microscope with shield in place (right panel)

Important Please ensure all components listed above were shipped to you. If not, please contact a 4Deep representative immediately.

2.4 Recommended Extras (not included)

The following is a list of recommended extra components, suggested by 4Deep, to optimize the use of your 4Deep Desktop microscope. Note that none of these are necessary for the use of the Desktop and the Desktop is fully functional without.

- A mm-capable ruler, used in Subsection 3.4.1 for proper Hologram reconstruction
- Compressed air canister
- Lint-free wipes/swabs (ex: Kim-tech), for use in cleaning (Section 4)
- LED-screen cleaner, for use in cleaning (Section 4)
- Cover for Desktop, for maintenance (keeping clear of dust when stored)

3 Installation and Use

The Desktop microscope system is completely assembled and requires only the supplied *power cable* and CAT5E c*ommunication cable* to operate immediately.

3.1 Connect the power cord

Plug the 12 VDC camera power cable into the outlet shown in Figure 3.1.

Figure 6: Power port shown as object “B” in the left panel. Plugging the power cable into the microscope port (right panel).

3.2 Connect to a Computer

Connect the microscope to the computer with Octopus software installed, with a CAT5 or 6 Ethernet cable, as shown in the left panel of Figure 7.

Figure 7: The Ethernet cable port on the microscope (left panel). Plugging the Ethernet cable into the microscope port (right panel).

Turn the laser on with the switch shown in Figure 8.

Figure 8: The power switch (object "A")

3.3 The Microscope and its Components

The Desktop microscope consists of 3 primary components:

1. Camera: Housed within the top portion of the microscope.
2. Sample Stage: An x-y-z translation stage
3. Point Source: A laser and pinholer/fibre optic cable, located in the bottom portion of the microscope.

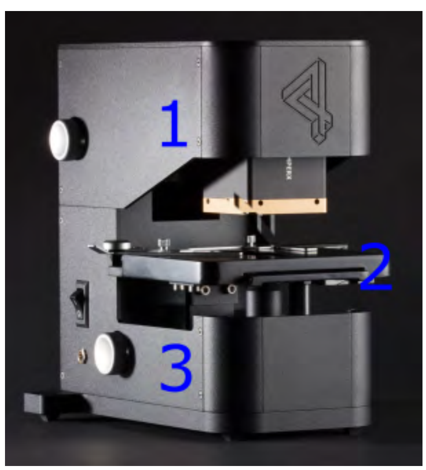


Figure 9: Main view of the Desktop microscope, showing 1) the camera housing, 2) the sample stage and 3) the point source

3.3.1 Camera

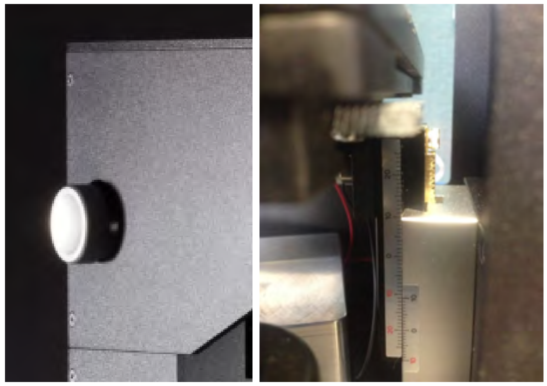


Figure 10: Camera vertical control knob (left panel) and the Source to Screeen Distance (SSD) scale (right panel)

The the digital camera included with the microscope is capable of 15 fps at full (2048x2048) resolution. The camera can be translated vertically, by the camera vertical control knob shown in the left panel of Figure 10. This knob moves the camera (thus the camera sensor) closer to/further from the point source, effectively altering the Source to Screen Distance (SSD). The SSD changes the magnification and resolution of the microscope via:

Increase SSD (move camera away from point source)	Magnification **Increases**	Resolution **Decreases**
Decrease SSD (move camera towards point source)	Magnification **Decreases**	Resolution **Increases**

Please see the Octopus software package for all camera software controls.

3.3.2 Sample Stage

The sample stage has two separate control knobs, one located next to the laser power switch which controls the vertical (z) translation stage shown in the left panel of Figure 11. This knob moves the translation stage up and down. Moving the stage up moves the sample (thus the objects in the sample) closer to the camera, and further from the point source (and vice versa), increasing the Source to Object Distance (SOD). The magnification of the microscope is dependent on the SOD, and the vertical position of the sample stage controls this.

Caution Be careful of the point source when moving the vertical sample stage position; **DO NOT** touch the point source.

Increase SOD	Magnification **Decreases**
Decrease SOD	Magnification **Increases**

Figure 11: The Z- control knob for the sample stage (object "C").

The second set of vertically aligned knobs is located on the other side of the microscope, which control the x-y translations of the stage, in the left panel of Figure 12. The top knob controls the full sample stage, and moves both the stage and holder (right panel of Figure 12), while the bottom knob moves just the sample holder (and any sample in the holder).

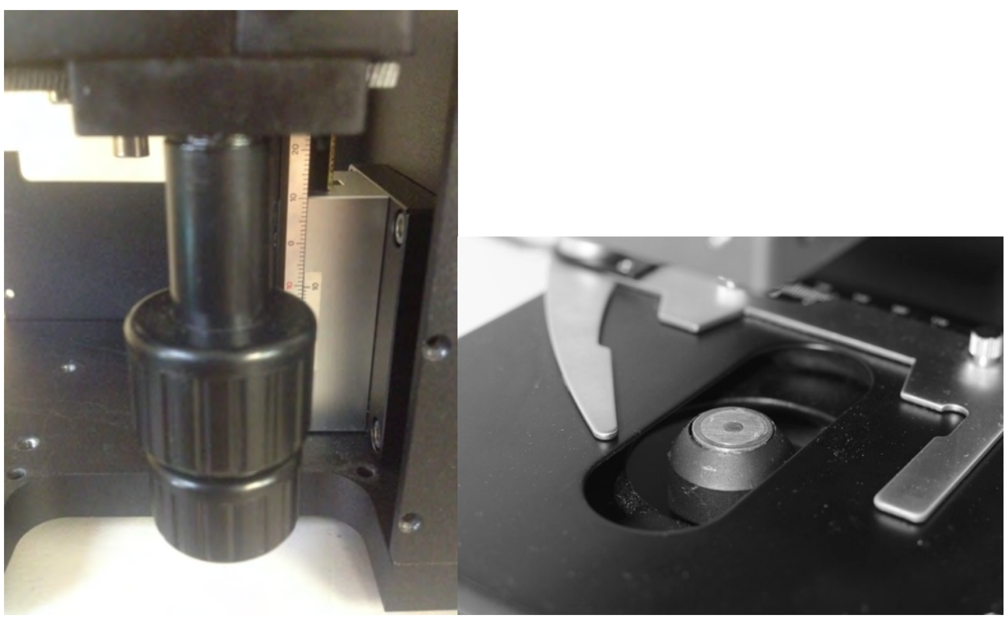


Figure 12: The x-y control knob for the sample stage (left panel). The top knob moves the full sample stage (along with the sample holder; right panel) in the x-y direction, while the bottom knob moves only the sample holder (metal slide holder in the right panel) in the x-y direction

The sample stage has several custom sample holders available, which are designed to hold standard microscope slides or sample dishes.

Important Be careful when handling the sample holder and sample itself. At the micron level, smudges on the holder can greatly affect the quality of the captured holograms. Take care not to smudge either side of the glass in the sample holder.

3.3.3 Point Source

Located in the lower portion of the microscope (Figure 13), the point source is made of an IIIB 405 nm laser, fibre optic cable near a micron in diameter (models 2014 - present). For older models (prior to 2014), instead of a fibre optic cable, a 60X objective, and a pinhole around 500nm was used.

Caution In earlier versions of the Desktop microscope (prior to 2014), the pinhole is exposed platinum foil shown, and is **extremely fragile.** Touching, blowing on or brushing this pinhole will be detrimental the the camera operation.

In newer versions (2014-present) of the Desktop microscope, the fibre optic cable is less fragile, however, it is still highly recommended to be very careful with handling the point source.

Important At the micron level, smudges/dust/dirt on the point source can greatly affect the quality of the captured holograms. Take care not to touch the point source.

Figure 13: Point source assembly

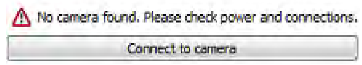
3.4 Collecting Holograms

To collect holograms, it is important to setup both the microscope and Octopus (4Deep's software) 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 Octopus for complete setup. You will also notice that much of the information listed below is also listed in the Octopus User Guide.

3.4.1 Acquisition setup for Octopus and the microscope

- Ensure that the sample holder, point source, and camera are all clean, free of dust, smudges, etc., as the presence of dust/dirt can greatly affect the quality of holograms, especially at the sub-micron level.
- Set up your sample on the sample stage by aligning the object/area of interest above the point source. You can align the sample using the controls for the sample stage (see Subsection 3.3.2)
- Start Octopus and click on the Camera Tab to connect Octopus to the camera (see Octopus User Guide for more details) .

- 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 5).



- 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.

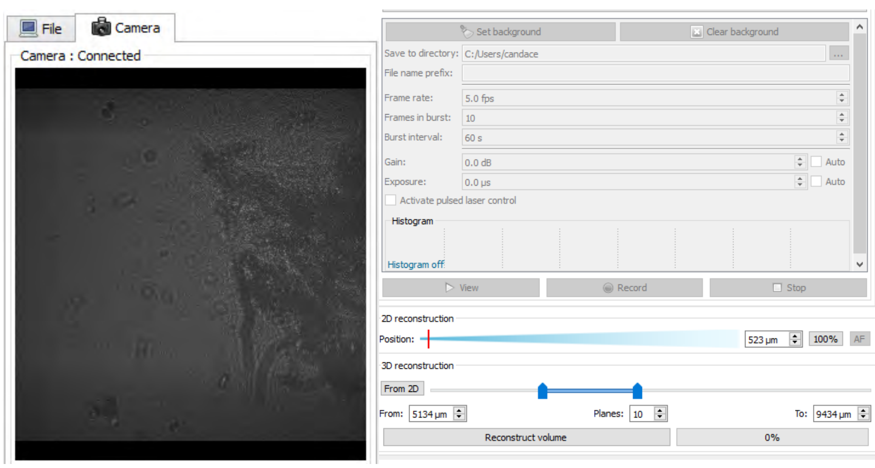
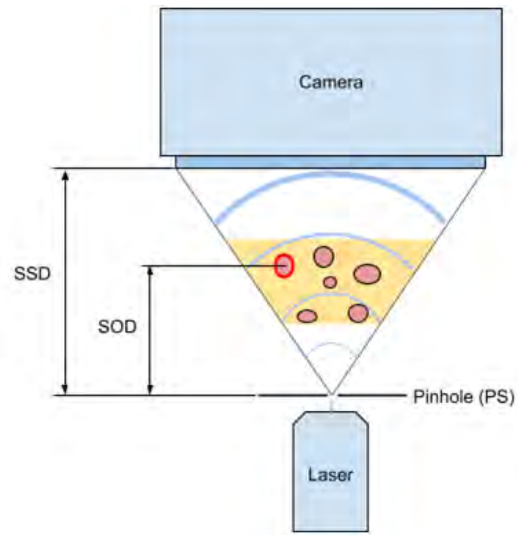


Figure 14: Camera tab view (left panel) and the camera options (right panel)

- Click the “View” button to start viewing (but not recording) the feed
- Once you can see the feed, you can change physical controls on the Desktop microscope to change the Source to Object Distance (SOD), as well as the Source to Screen Distance (SSD), which will help in hologram acquisition.
  - The SSD distance is controlled by moving the camera control knob (see Subsection 3.3.1) . The goal of changing the SSD is to have even illumination in the camera view. The optimal distance for the Desktop microscope should be around 30-40 mm.



- Image resolution is dependent on the SOD. As this distance decreases, the resolution and the magnification increase. Maximum resolution is obtained by placing the sample/object as close as possible to the Point Source (PS). However, keep in mind that as the SOD distance decreases, the field of view becomes smaller (see Subsection 3.3.2).

- Once the microscope is physically set up, use a ruler (with mm capability) to measure the distance from the bottom of the camera housing to the top of the sample stage. By adding this number to 3.3mm (distance the screen is located within the the camera housing), you will have calculated the source to screen distance (SSD)

- To properly reconstruct holograms, settings including the SSD need to be added to Octopus.

  - Select the Preset Option for the Desktop: Settings -> Reconstruction Options-> Preset. Then, if the SSD is not the same as value as the "Laser to camera distance", change it to the one you measured above. If you have changed the SSD, then, 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.

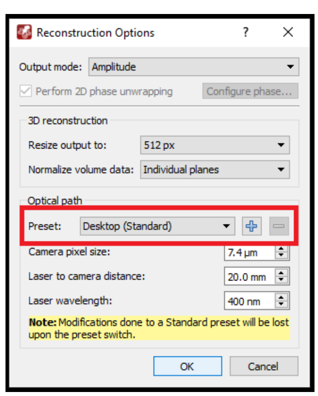


Figure 15: Select instrument preset dialog

Caution Go to Octopus User Guide for further details on capturing holograms (Section 4 in the Octopus User Guide).

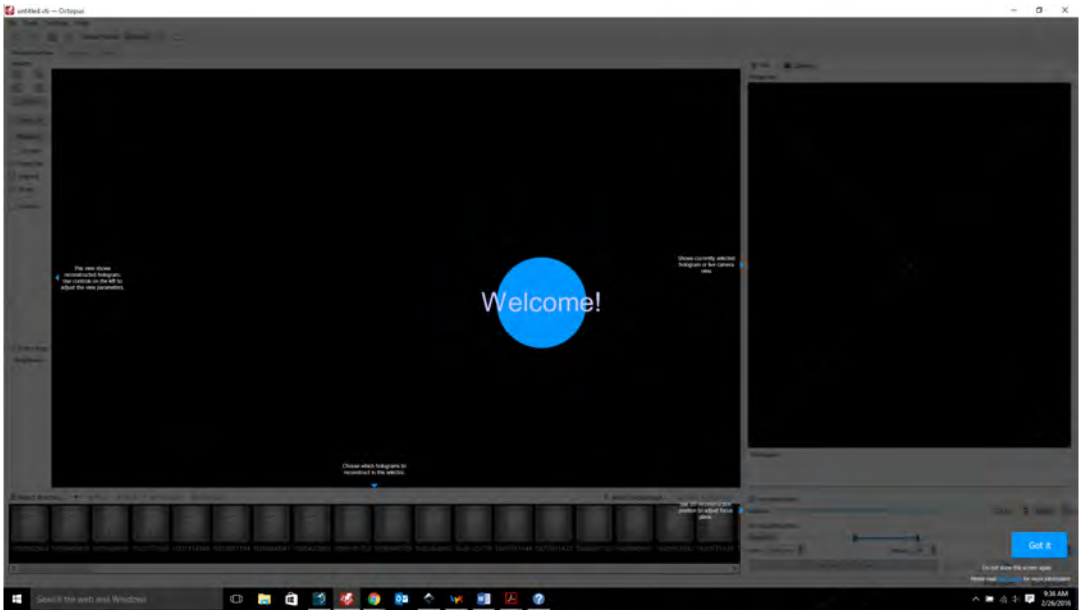


Figure 16: The Octopus software at start-up

3.4.2 Tips for Optimal Hologram 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 of the Octopus software.

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.

Further information regarding hologram acquisition can be found in the Octopus User Guide.

4 Care, Cleaning and Maintenance

Your Desktop microscope requires very little maintenance:

- Keeping the microscope **casing** clean and free of lint or dust is recommended.
- Storing the microscope protected from dirt, dust and moisture will ensure a long operational life.
- **Take all measures to prevent contact with the point source, as any dust or liquids on the point source may prevent the proper operation of the microscope.**

You can clean dust and dirt off the microscope with a compressed air duster.

Caution Ensure that the air duster is safe for plastics and optics.

The glass plate protecting the camera (in the camera housing, facing down) should only be cleaned when absolutely necessary. If you are unsuccessful in cleaning the camera with the air duster, please contact 4Deep for help.

The point source should also only be cleaned of smudges when absolutely necessary. The point source can be cleaned with an LCD screen safe cleaner, and a lint-free swab. Ensure that when you clean the point source, you clean it gently with light strokes, as scrubbing it may ruin the finish on the fibre.

5 Troubleshooting

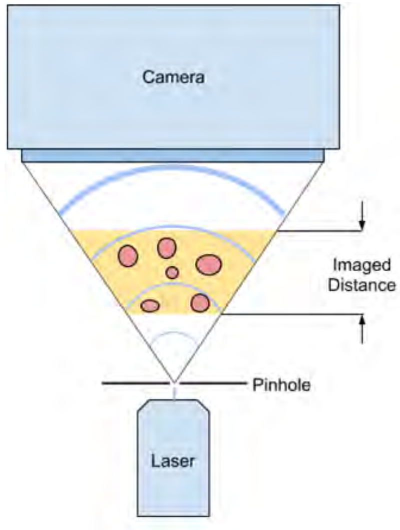
Issue	Solution
Camera not connecting in Octopus	Check that the cables are properly attached, and power is on If there are more than one Ethernet ports on the computer, try a different one If you are using a laptop computer docking station, use the Ether net port on the docking station (not the laptop itself)
No light from point source/ not much light from point source	If there is no light coming from the point source and it is properly connected there may be an alignment problem with the laser, there may be direct damage to the point source, or the laser diode may have burned out. Send the microscope or point source back to the manufacturer for re-alignment. Background light must be reduced to obtain optimal images. Try using a light shield (standard with Desktop microscope)

Table 1: Common troubleshooting problems and solutions

6 Appendix

6.1 Principle of Operation

The Desktop microscope operates on the principles of holography to image a volume in magnification. A 405 nm laser from a fibre optic cable produces a spatially coherent light source as a reference wave. Light scattering from the objects within the media (water) will interfere with the reference wave to produce an interference pattern which contains spatial and phase information of the objects within the volume. This interference pattern, the hologram, is recorded by a CCD camera, and reconstructed mathematically to build images of the objects within the volume.



The basic principle of digital in-line holographic imaging

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

6.2 The Advantages of Holographic Microscopy

The images obtained from holographic microscope 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.

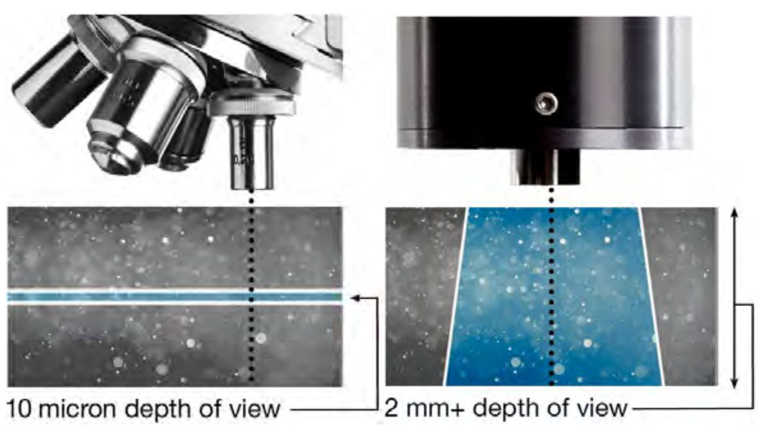


Figure 17: Field of view comparison of lens based microscope (left) and holographic microscope (right)

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.

6.3 Software

The holograms acquired by the Desktop microscope camera need to be properly processed in order to reconstruct the image.

4Deep provides 2 standard software packages, *Swordfish*, *Octopus*, and *Stingray*, which incorporate our patented fast hologram reconstruction algorithm as well as additional functionality.

Swordfish is a particle counting software capable of detecting, counting and measuring particles in real time with the speed of up to 15 frames per second.

Octopus is a general purpose research-grade software package that can reconstruct holograms acquired by the microscope camera at the rate of up to 15 frames per second. Octopus provides advanced 3D visualization and particle detection capabilities.

Stingray is an automated particle recognition and morphology classification software platform. It develops a user defined database from which it learns the type of objects to be detected.

Please refer to the Swordfish, Stingray and Octopus brochures and manuals available at www.4-deep.com for the additional details on software installation and use.

Custom software solutions can be developed by 4Deep based on customer specifications. Contact us for details.

7 Specifications

Resolution	1.2 micron
Power	5.5 Watts
Footprint	22 cm x 18 cm
Weight	5 kg
Data transfer	Gigabit Ethernet
Computer operating system	Windows XP SP3 or later
Communication & power	Ethernet & 12 VDC
Hologram size	from 2048x2048 to 512x512
Frame rate	from 16 to 50 fps
Field of view	Up to 5mm
Imaged object size	1μm-3mm
Input voltage (provided)	12 VDC