4Deep
inwater imaging

Submersible Microscope User Guide

Version 1.3.0

Notices

The Submersible microscope needs to be cleaned after EVERY use with window cleaner.

Edition

Submersible User Guide - Version 1.3.0

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.

Submersible Microscope Version 1.3.0

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1 Overview

The Submersible Microscope has been developed to operate in conjunction with 4Deep's software (Octopus, Swordfish or Stingray). 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 Submersible microscope. For a detailed description of the setup and operation of the 4Deep software, please refer to the respective user guides.

The Submersible Microscope is an in-water microscope; for the benchtop microscope, see the Desktop Microscope.

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

- Marine research: water profiling, algae, plankton, phytoplankton
- Oil & gas
- Water quality and monitoring: microorganism imaging
- Ocean research
- Algae production: algae profiling
- Counting, data and morphological analysis, quantitative phase analysis: many other applications

1.1 Purpose

The Submersible 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 Submersible Microscope allows you to quickly and easily observe micro-organisms and particles up to a depth of 5 kilometres (in custom models).

The measurement technique used is digital inline holographic microscopy in transmission. The light source is a single source of 405 nm.

1.2 Benefits

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

- Real-time, in-situ capability to observe of particles and micro-organisms in all aquatic environments
- Portable and lightweight, making it easy to deploy, yet rugged
- High-flow capabilities; an be towed at 8 knots (4 m/s), or stationed in rivers
- Low maintenance and easy to clean, as there is no pumps or tubing, making fouling less of an issue

- Fast-tracking: up to 22 images per second to capture tracking dynamics of fast moving objects
- Save time using the fastest hologram image reconstruction available
- Go deeper, as it is pressure rated from 0-500m, with a cable depth of 100m, and Up to 6000 meters in custom models
- Instant: live imaging of samples without any manipulation.
- 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. A 405 nm wavelength is emitted from the laser housing itself. If properly assembled, the light emitted from the laser 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 Submersible microscope needs to be used in conjunction with the Octopus software. The computer requirements are:

- CPU Core i5 or i7
- 8 GB of RAM
- 256 GB or larger SSD Drive
  - The microscope can record at 22 images per second, and each image is 4 MB
- NVIDIA GeForce or Quadro GPU with at least 2 GB of graphics RAM, Kepler architecture or later
  - 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
- Gigabit Ethernet port (for data connection)

- 1 USB port for the HASP key -Full versions of the software require a valid HASP key (dongle) to run
  - Demo versions can be used without a HASP key
- Windows 7 or 10, 64-bit
  - Windows 10 Pro is preferred over other versions (such as Home)

2.3 Components of the System

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

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

- Submersible microscope, pre-assembled. For full details, see the microscope description in Section 2.5.
  - There are two purge valves on the bottom of the microscope, as shown in the right panel of Figure 1
  - Caution **DO NOT REMOVE THE PURGE VALVES**. Using the purge valve without the advice of 4Deep **WILL NEGATE ANY WARRANTY** on the microscope. Purging of the microscope MAY only be needed in one specific issue, thus in most cases, your microscope will NOT need to be purged.

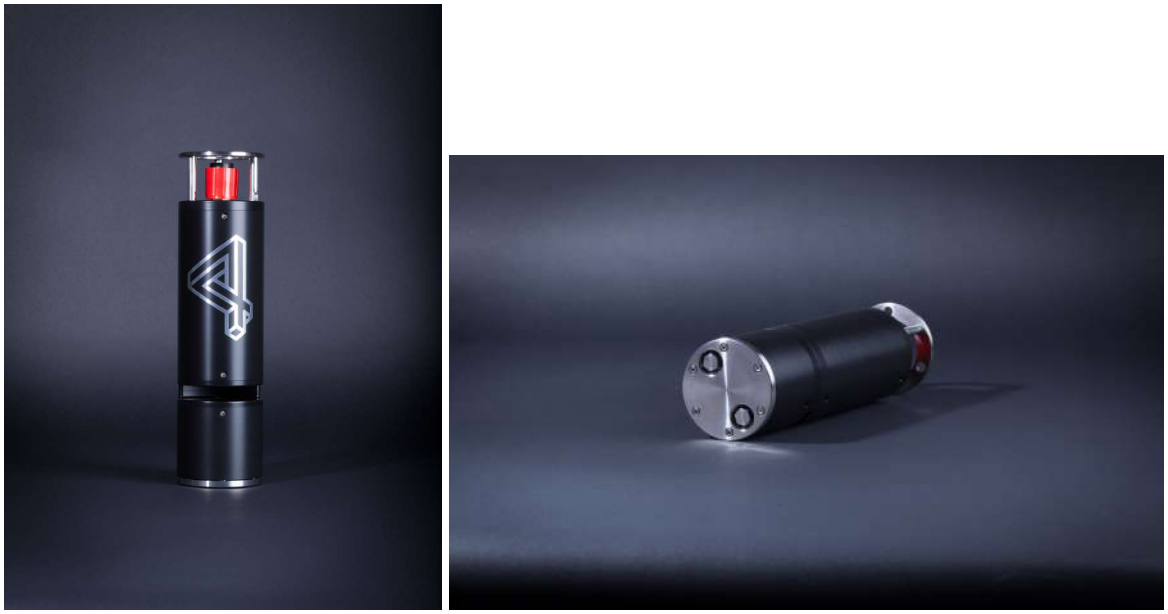


Figure 1: The preassembled Submersible (vertical view in left panel and horizontal view in right panel).

- Long (10m) cable with 13 pin connector to power laser, camera, and transmit data from the camera to the host computer.

Figure 2: 10m Cable

- Power supply (12 VDC)

Figure 3: Power Supply (12 VDC)

- Carrying case

- Flow-through device (optional)

Figure 4: Carrying case, with the microscope inside

Figure 5: Flow-through chamber

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 Submersible microscope. Note that none of these are necessary for the use of the Submersible and the Submersible is fully functional without.

- Molykote 44 Medium, for marrying the connectors (Subsection 3.1)

- Deployment rope/cord, to deploy the Submersible (Section 3.4)
- A female-female Ethernet connector (ex: from a computer hardware store, shown in Figure 13)
- Lint-free wipes/swabs (ex: Kim-tech), for use in cleaning (Section 4)
- Glass/window cleaner, for use in cleaning (Section 4)

2.5 The Microscope and its Components

The Submersible microscope consists of 5 primary components:

1. Chamber 1: houses the laser point source
2. Chamber 2: houses the camera
3. Sample Space: connects the two chambers, determines the sample volume that will be imaged.
4. Crown
5. Purge Valves

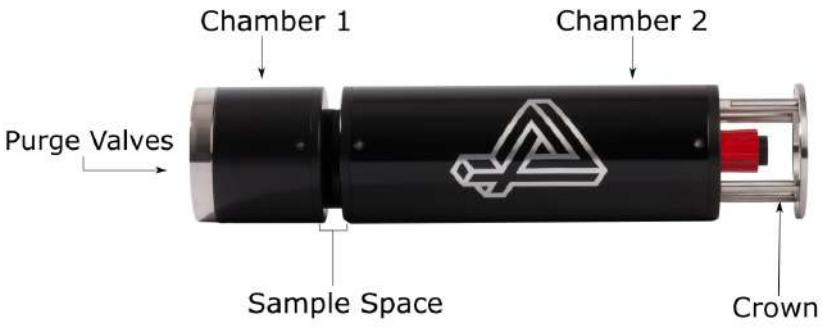


Figure 6: Submersible Microscope components

2.5.1 Chamber 1

This Chamber contains a digital camera. The camera's image sensor is protected by a sapphire glass window. The sapphire window also has a copper ring around it, to reduce the effect of biofouling on the instrument (shown in Figure 7).

Caution This window is the most fragile external component of the microscope, and must be handled carefully.

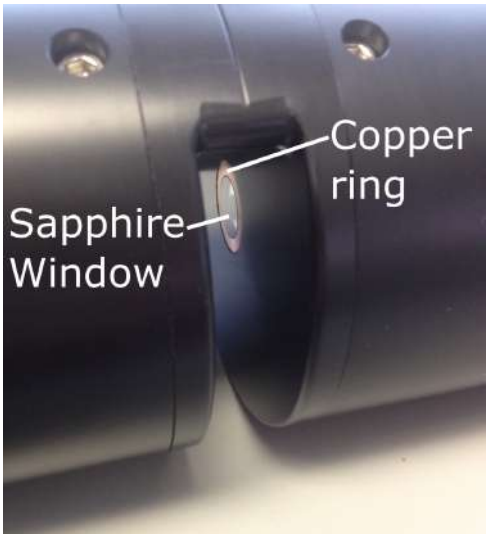


Figure 7: Protective sapphire camera window and copper ring.

Important Be careful when handling the microscope. At the micron level, smudges on the windows can greatly affect the quality of the captured holograms. Take care not to smudge the windows.

2.5.2 Chamber 2

This Chamber contains the laser point source, consisting of a 405 nm laser and a fibre optic cable, which serves as a point source. The point source provides the spherical wave of light necessary to produce images in inline holography. Similar to the camera in Chamber 1, the point source is protected by a sapphire glass window (see previous paragraph for care instructions).

2.5.3 Sample Spacer

The Sample Spacer provides a stable environment for imaging microorganisms and particles in addition to determining the source to screen distance of the instrument, which is essential for proper data analysis.

The Sample Space, determined by the Sample Spacer, allows for the non-destructive flow of organisms and particles. The Submersible Microscope's camera collects holograms of the

samples as they, along with the fluid which contains them, pass through the Sample Space. The standard Sample Space for a 4Deep Submersible is 15 mm.

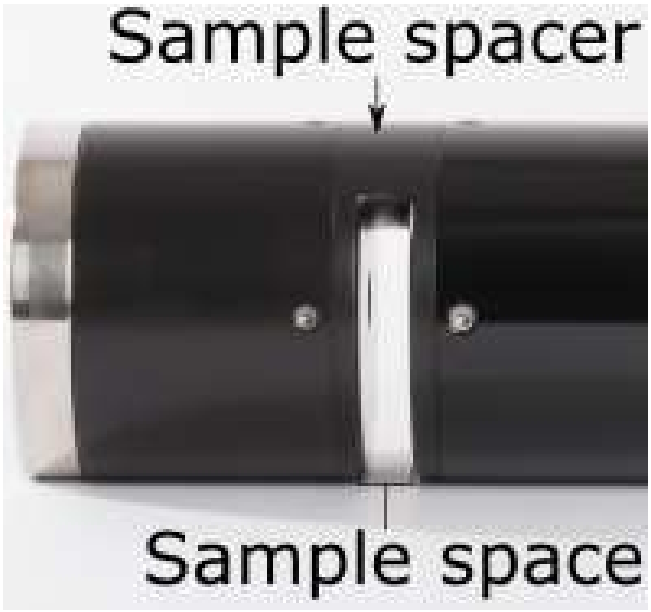


Figure 8: Sample space

2.5.4 Crown

The Crown is the metal framing around the connector on the Submersible microscope. Its main purpose is for handling and deployment.

2.5.5 Purge Valves

There are two purge valves on the bottom of the microscope, as shown in the right panel of Figure 9.

Figure 9: The two purge valves on the bottom of the microscope.

The purge valves do not need to be used to ensure the microscope operates correctly. If a 4Deep representative deems it necessary for the purge valves to be opened, it will be completed at the 4Deep office, **NOT BY THE USER.** Note that using the purge valves is only necessary to solve one issue that MAY occur with the microscope, so it is likely the purging will not need to occur.

Caution **DO NOT USE THE PURGE VALVES**. Using the purge valve without the advice of 4Deep **WILL NEGATE ANY WARRANTY** on the microscope.

3 Installation and Use

3.1 Connect the Submersible cable

Firstly, ensure the connector pins (male and female) are clean, and free of any dirt/dust before connecting.

The connector pin manufacturer suggests using Molykote 44 Medium grease before every mating (Figure 10). The manufacturer suggests greasing about 1/3 of the female socket depth (right panel of Figure 10), for a “wet mating” (ie if the connector/microscope is being submerged). The manufacturer suggests greasing about 1/10 of the female socket depth , for a “dry mating” (ie if the connector/microscope is not being submerged, for example, when using the microscope in a laboratory environment with the flow through collar).

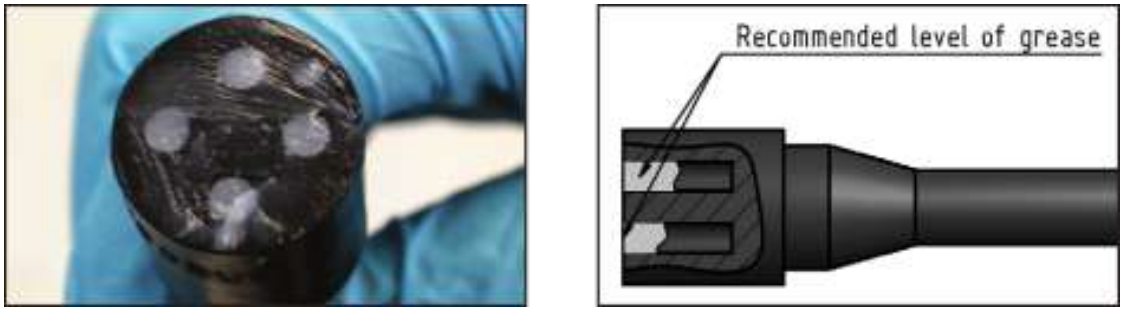


Figure 10: Mating the underwater connectors for a wet mating (Photo from MacArtney Underwater Technologies)

Caution As you push the connectors together, take care not to wiggle the cable too much, as it may cause damage the pins.

Firmly push the 13 pin connector at the end of the main underwater cable into the bulkhead connector. Push the cable connector all the way in. To verify that all of the male connectors are greased, pull the connectors apart, and check that the male pins are all coated in grease. Then reconnect the cable.

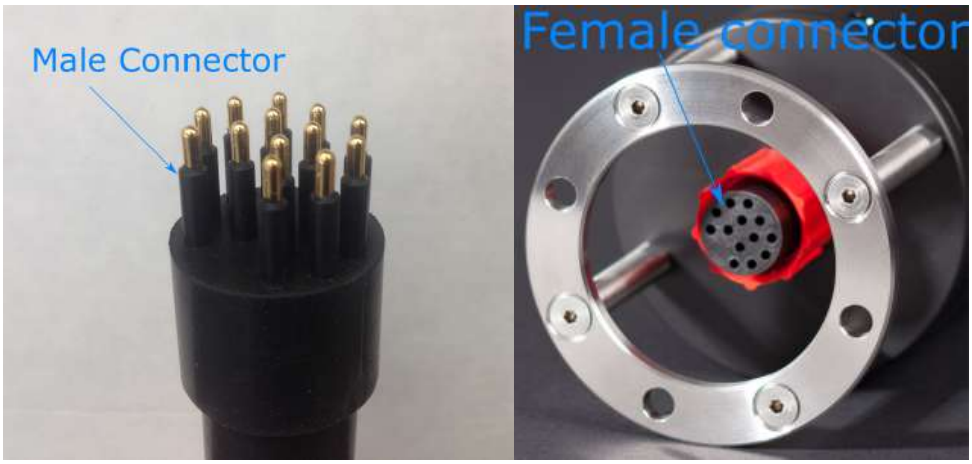


Figure 11: Submersible Microscope bulkhead with 13 pin connector. The male connector is in the left panel, and is attached to the underwater cable. The female is shown in the right panel and is attached to the microscope.

3.2 Screw on locking sleeve

Slide the locking sleeve over the cable connectors and screw it onto the bulkhead connector.

Figure 12: Locking sleeve on both the cable (left panel), the microscope (center panel) and connected (right panel)

3.3 Ethernet Connection

Caution It is highly recommended that you use a flexible Ethernet cable, and a female-female Ethernet connector as the underwater cable is very rigid and may damage the Ethernet port on your computer.

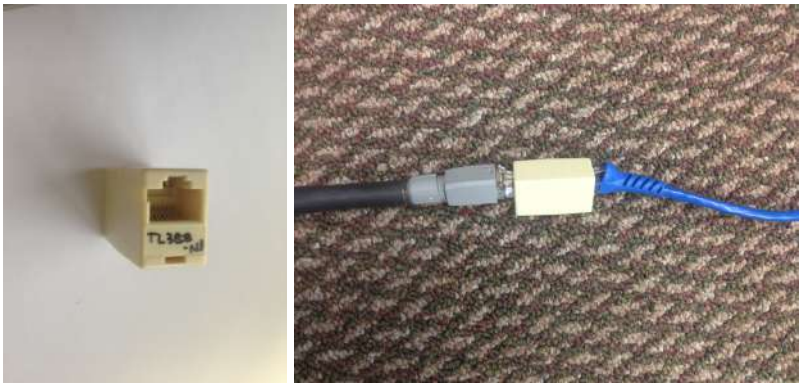


Figure 13: Use of female to female Ethernet connector. In the right panel, the black cord is the rigid Ethernet cable from the underwater cable, and the blue cable is the flexible Ethernet cable to the computer.

- Connect the Ethernet end of the main cable to the Ethernet port on the computer.

Figure 14: Connecting to the computer

- Plug in the power adapter for the camera and plug in the laser power adapter to the connector on the same cable.

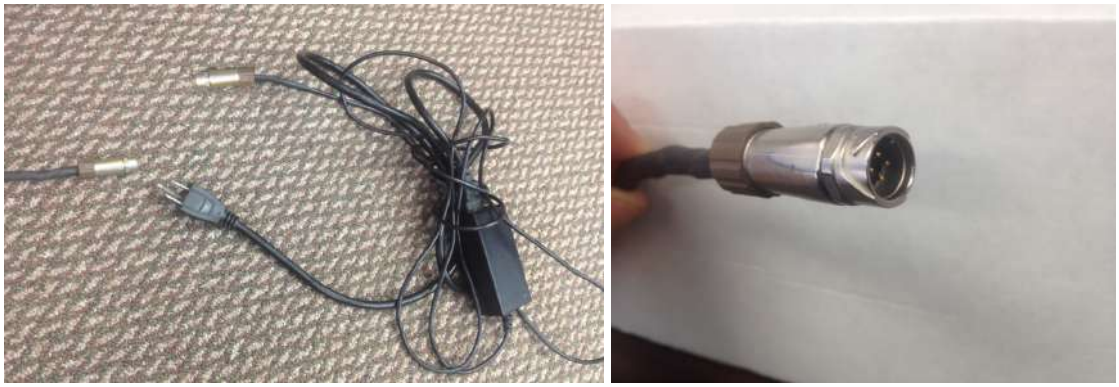


Figure 15: Connecting the power. In the left panel connecting the microscope power to the included 12VDC power cord. The right panel shows a close-up of the power connector from the microscope.

3.4 Testing and Deployment

Many applications of the Submersible will require deploying the Submersible into a body of water (river, ocean, etc.) off a platform (boat, wharf, etc.).

Caution DO NOT deploy the Submersible with the underwater cable alone. Attach a rope to the crown atop the Submersible microscope for deployment. Submerse the microscope into the body of water/fluid that you want to look at. Both sapphire windows should be fully submersed in water for the accurate imaging to be performed.

Figure 16: Deployment of the Submersible from a wharf

More complex deployment methods may also be necessary, as researchers may wish to deploy the Submersible microscope in conjunction with other instruments. As this would be application-specific, we leave the details of the deployment up to the user, but give the dimensions of the Submersible system for technical purposes:

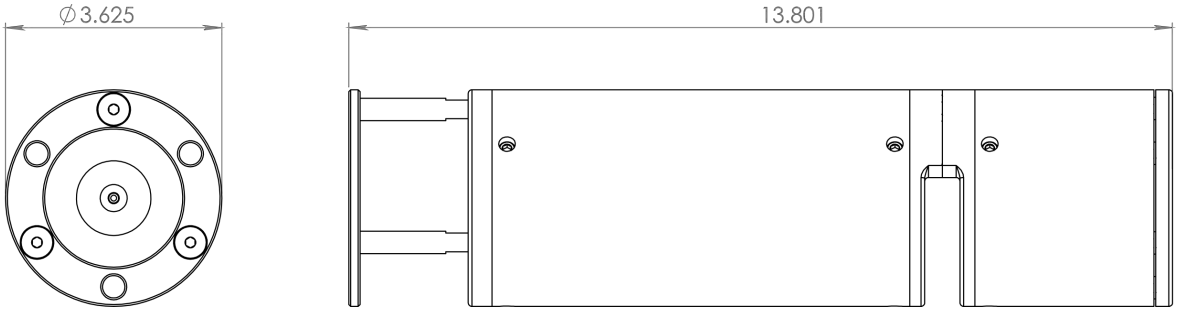


Figure 17: Dimensions of the Submersible, reported in inches

3.4.1 Flow-through Chamber

If the flow-through chamber is used:

1. Important Double check that o-rings are in place. The flow through collar should be delivered with the o-rings in place
    - If the o-rings are not properly in place, the flow through collar will leak
2. Important Dampen the o-rings using a small amount of water, applied by your finger
    - If the o-rings are not lubricated, the force of pushing the flow through collar in may dislodge the o-rings, making the flow through collar leak
    - If too much water is used to lubricate the o-rings, it will be hard to verify it is sealed, so do not spray water on the o-rings or immerse them in water to lubricate

3. Firmly push the flow through collar into the microscope
    - The flow through collar should slide in easy if lubricated properly
4. Tubing will be needed to use the flow through collar. It is recommended that low-pore tubing, such as teflon tubing, is used
5. Ensure that the tubing used does not go onto the hexagonal part of the nipple
    - The hexagonal part is only for wrenches. If the tubes go over those parts, the tubes won't be sealed, so the tubes will leak.
6. If changing the nipples on the flow through collar:
    - Do not over-tighten the nipples. Hand tighten the nipples, and then only tightened with a wrench no more than ¼ turn
    - Ensure the correct-sized tubing is used for the different nipples

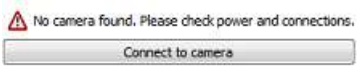
Caution When the tubing is cleaned, ensure it is rinsed thoroughly before re-attaching to the Submersible microscope, to ensure any cleaners do not contact the microscope.

3.5 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.

3.5.1 Acquisition setup for Octopus and the microscope

- Ensure that the point source and camera are clean, free of dust, smudges, etc., as the presence of dust/dirt can greatly affect the quality of holograms, especially at the micron level.
- 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.

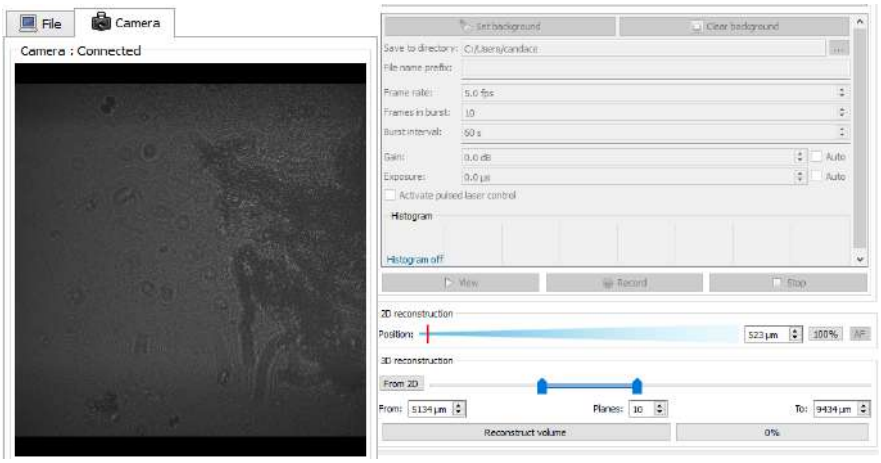


Figure 18: Camera tab view (left panel) and the camera options (right panel)

- Click the “View” button to start viewing (but not recording) the feed
- To properly reconstruct holograms, settings including the source-to-screen distance need to be added to Octopus.
  - Select the Preset Option for the Submersible: Settings -> Reconstruction Options-> Preset.

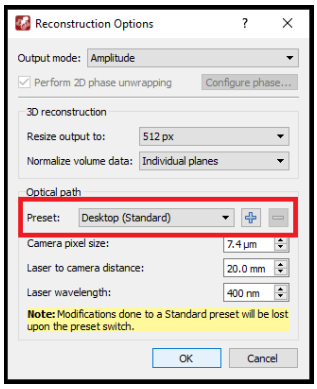


Figure 19: Select instrument preset dialog

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

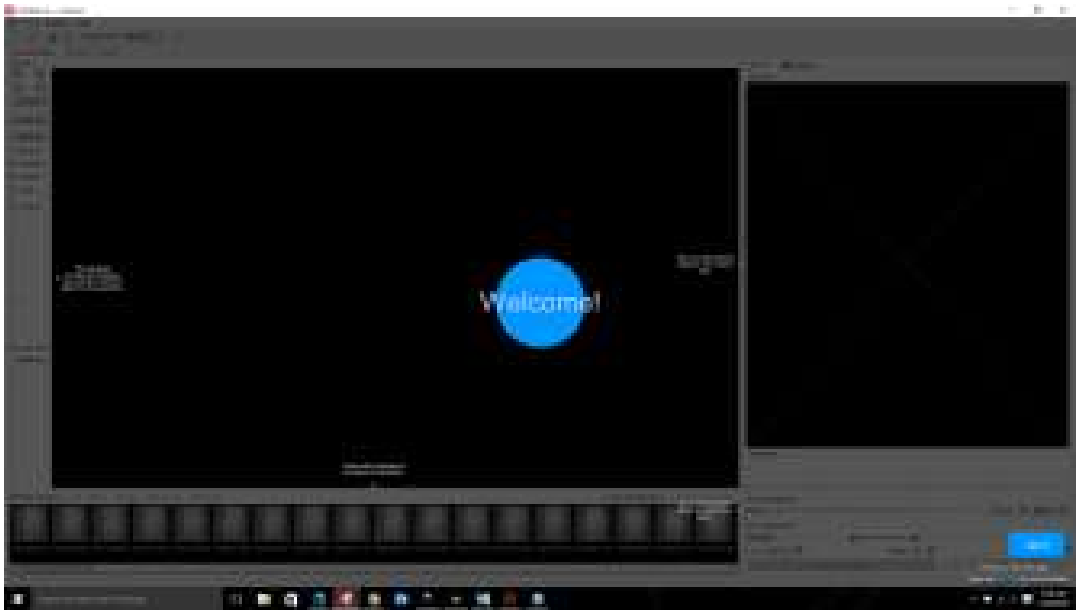


Figure 20: The Octopus software at start-up

3.5.2 Tips for Optimal Hologram Acquisition

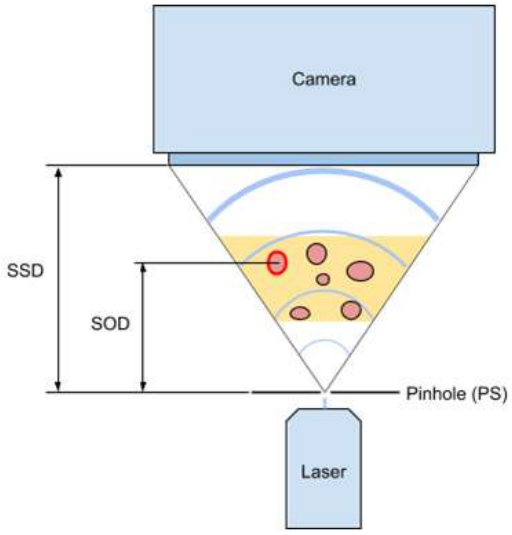


Image resolution is dependent on the SOD (Source to Object Distance). As this distance decreases, the resolution and the magnification increase. Maximum resolution is obtained by placing the sample/object as close as possible to the point source. However, keep in mind that as the SOD distance decreases,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

The distance between the camera sensor and the point source - Source to Screen Distance (SSD) in the Submersible microscope is fixed, based on the configuration of the microscope. The standard configuration provides 54 mm SSD, and offers 15 mm of imageable space. Contact 4Deep for the development of custom configurations for special applications.

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.

If possible, always 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.

4 Care and Maintenance

While the maintenance of your Submersible microscope is minimal, some cleaning is required.

1. You microscope does NOT have to be cleaned for the first deployment. Just ensure the sapphire windows are free of dust/dirt by connecting to a computer (see Section 3)
2. Before EVERY deployment the Submersible microscope connectors should be mated with grease (see Subsection 3.1)
3. After EVERY deployment the Submersible microscope needs to be washed in clean fresh water to minimize the potential for corrosion.
4. After EVERY deployment the Submersible microscope needs to be dried off before being replaced in the carrying case. This includes using an air duster to blow water away from crevices in the microscope.
5. After EVERY deployment the sapphire windows need to be cleaned with window cleaner and lint-free wipes. Note that while the sapphire glass is resistant to scratches, the reconstruction of holograms is very sensitive to dust/dirt in the field of view. Therefore, when cleaning take care to remove all dust and dirt.
6. Avoid transporting the microscope along with loose hardware (Allen keys, screws, etc) as they will scratch the sapphire window surface if contact is made.

Caution Without cleaning the microscope, there will be an accumulation of particles on the sapphire windows, which will destroy the quality of the holograms and make the data useless.

5 Troubleshooting

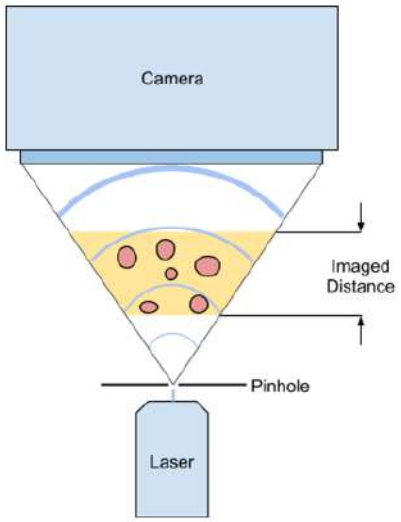
Issue	Solution
Camera not connecting	Check that the cables are properly attached, and power is on If there are more than one Ethernet ports on the computer, try a different one If you are using a laptop computer docking station, use the Ethernet port on the docking station (not the laptop itself)
No 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 a problem with the laser, there may be direct damage to the laser, or the laser diode may have burned out. Send the microscope or point source back to the manufacturer for diagnosis. Background light must be reduced to obtain optimal images.
Flow through collar is leaking	Verify the o-rings are in place. Ensure the o-rings are dampened before inserting the flow through collar into the microscope Once all other options are used, replacing the o-rings may be required

Table 1: Common troubleshooting problems and solutions

6 Appendix

6.1 Principle of Operation

The Submersible 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 Software

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

4Deep provides 3 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 10 frames per second.

Octopus is a general purpose research-grade software package that can reconstruct holograms acquired by the Submersible microscope camera at the rate of up to 22 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

Optical Resolution	1.5 microns
Laser pulse rate	0.5 µs
Tow rate	8 knots
Point source wavelength	405 nm
Power	< 5 Watts
Power requirement	120/240 VAC
Communication	13 pin SeaConn Ethernet connector
Data transfer	Gigabit Ethernet
Computer operating system	Windows 7 or later
Hologram size	2048x2048
Frame rate	22 fps
Sample space	15 mm
Sample volume	up to 100 microlitres
Size	92mm x 351mm
Weight in air (water)	2.6 kg (0.5 kg)