4Deep
inwater imaging

Cuvette Microscope User Guide

Version 1.0.0

Notices

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Edition

Cuvette User Guide - Version 1.0.0

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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 Cuvette 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 Cuvette microscope. For a detailed description of the setup and operation of the 4Deep software, please refer to the respective user guides.

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

- Cell Biology
- Microbiology
- Cancer Research
- Blood / urine collection
- Exocytosis
- Marine research: water profiling, algae, plankton, phytoplankton
- Water quality and monitoring: microorganism imaging

1.1 Purpose

The Cuvette 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 Cuvette 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.

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 150 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. use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.**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

If you did not purchase a computer directly from 4Deep with pre-configured software, here is the required *minimum* specifications:

- Windows XP SP3 or newer (7 or 8 recommended)
- Intel Core i5 or better CPU
- 4 GB of RAM
- NVIDIA graphics card, CUDA 6.0 compatible
- Gigabit Ethernet port
- 500 GB + available space on the hard drive for storing holograms

2.3 Components

The following is the list of parts shipped as part of the Cuvette microscope.

1. Cuvette microscope, pre-assembled.

Figure 1: Cuvette microscope

2. Power supply (12 VDC)

Figure 2: Power supply

3. Ethernet cable

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

4. Two quartz sample cuvettes. The microscope uses standard (12 mm x 12 mm x 45 mm)

laboratory spectroscopic quartz cuvettes as sample chambers.

Figure 4: Cuvette samples

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

- Compressed air canister
- Lint-free wipes/swabs (ex: Kim-tech), for use in cleaning (Section 4)

3 Installation and Use

The Cuvette 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 as Object A in Figure 3.1.

Figure 5: Power connection

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 Object B of Figure 3.2.

Figure 6: Ethernet connection

3.3 Place the sample in the Microscope

Ensure that the quartz cuvette, 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. Place desired sample in a clean cuvette (for best results, always wear gloves when handling the quartz cuvettes), and place in the cuvette well of the microscope.

Figure 7: Cuvette for microscopy

3.4 Turn the power on

The power button lights up when the power to the microscope is on. The “up” position means the Cuvette microscope is on.

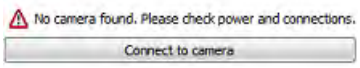
Figure 8: The Power button

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

- 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.

- Click the "View" button to start viewing (but not recording) the feed

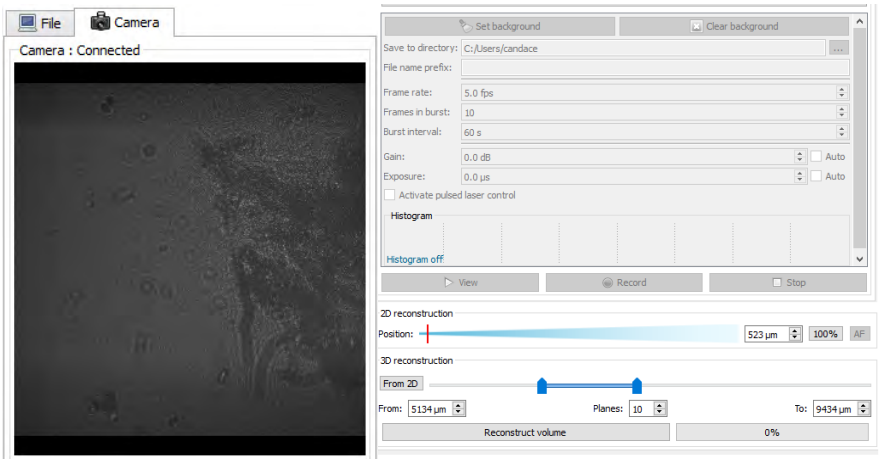


Figure 9: Camera tab view (left panel) and the camera options (right panel)

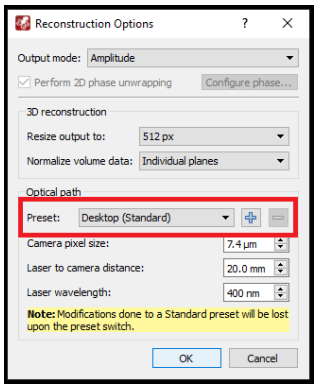


Figure 10: Select instrument preset dialog

- Select the Preset Option for the Cuvette microscope: Settings -> Reconstruction Options-> Preset.

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

3.5.2 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.

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

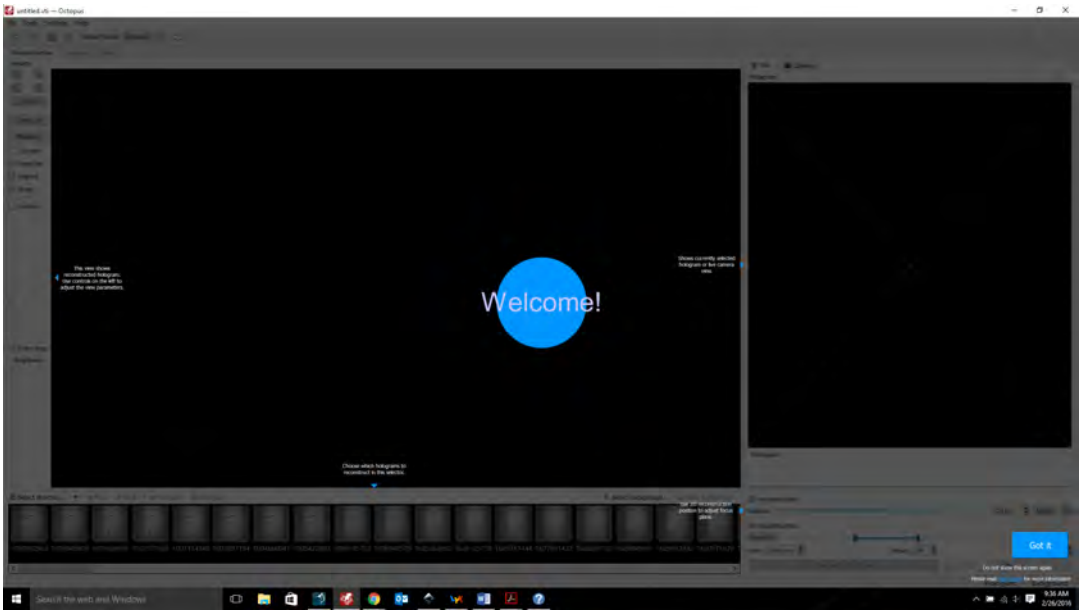


Figure 11: The Octopus software at start-up

background subtraction when reconstructing holograms, producing higher contrast reconstructions while reducing noise.

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

4 Care and Maintenance

Your Cuvette microscope requires very little maintenance.

- It is recommend that after *every* experiment the sample cuvette is washed in clean fresh water to minimize the potential for fouling. Quartz cuvettes are recommended as they give the clearest images, and do not scratch easily with use and cleaning.
- Store the Cuvette microscope with the window closed to ensure no dust/dirt is introduced when it is stored.

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 covering the sample with something to block out the light.

Table 1: Common troubleshooting problems and solutions

6 Appendix

6.1 Principle of Operation

The Cuvette microscope operates on the principles of holography to image a volume in magnification. A 405 nm laser shines through a small aperture, which 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 reconstructed images can be further analyzed, saved, or assembled into the volume reconstructions based on the application.

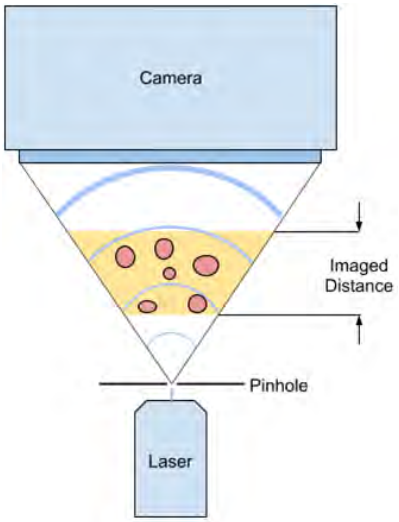


Figure 12: The basic principle of digital in-line holographic imaging

6.2 Software

The holograms acquired by the Cuvette 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 16 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 16 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	2 micron
Power	3 Watts
Size	20 cm x 10 cm x 5 cm
Weight	0.5 kg
Data transfer	Gigabit Ethernet
Computer operating system	Windows XP or later
Communication & power	Ethernet & 12 VDC
Hologram size	1024x1024
Frame rate	up to 60 fps
Sample space	10 mm (cuvette)
Sample volume (1 hologram)	5 microlitres