

HoloScan User Guide



Version 1.0

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1 Overview

The HoloScan is a hardware + software solution to scan liquid-sampling membrane filters, to determine if the fluid is contaminated with bacteria and/or yeast.

Note that throughout this user guide, the hardware will be referred to as the “HoloScan hardware” or “microscope”, and the software will be referred to as the “HoloScan software”. Any mention of the “HoloScan” refers to the whole system.

To ensure that high resolution images of bacteria and yeast are collected, the filter is scanned in small sections by the software. Each step of the scan captures a hologram of a section, ensuring the entire filter is sampled. Once completed, the holograms are analyzed in 4Deep’s other software packages to determine if there are bacteria or yeast present.



Figure 1: HoloScan hardware.

2 HoloScan software Installation Guide

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 and 48 VDC power supplies are used with the microscope. Failure to do so could result in critical damage to the microscope.

Note that on the back of the HoloScan software the following Caution is also present:

Caution CLASS IIIB VISIBLE AND INVISIBLE LASER RADIATION WHEN OPEN AVOID EXPOSURE TO BEAM

2.2 Compatibility

The following are the computer requirements for the HoloScan software:

- CPU Core i5 or i7.
- 8 GB of RAM.
- 256 GB or larger SSD Drive –
 - The HoloScan hardware can record at sub-second speeds, and each image is 4 MB, leading to a large database, thus storage may be an issue.
- The best GPU available: NVIDIA GeForce 1080 or NVIDIA Quadro P5000 (or better) with at least 2 GB of graphics RAM, Kepler architecture or later.
 - For fast processing, 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).
- Recorded and reconstructed images are exportable as PNG.

2.3 Installation Package

To install the HoloScan software on your computer

- Download the HoloScan software from our website: <http://4-deep.com/software-downloads/>.
- Insert the HASP key supplied and follow the onscreen instructions.
- Note that for fast processing, 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 the HoloScan software by running HoloScanInstaller.exe and following the onscreen instructions. Selecting the default parameters should typically be acceptable for most installations.

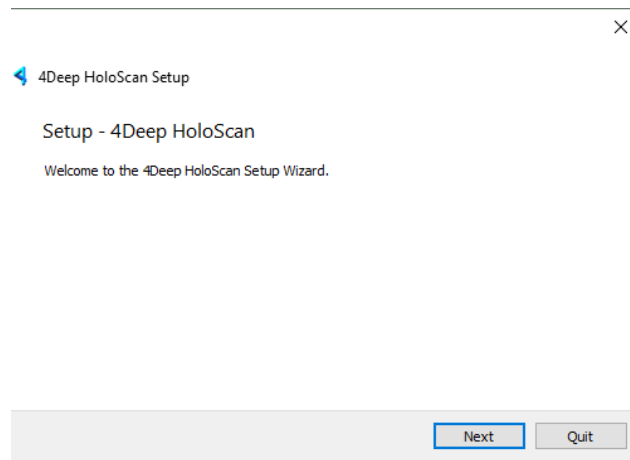


Figure 2: HoloScan software installer.

2.4 Ethernet settings

Processing in the HoloScan software relies on the graphics card in the computer, thus the system settings on Windows need to be optimized.

- Navigate to the Network Connections. In Windows 10, via [Control Panel -> Network and Sharing Center -> Change adapter settings -> Ethernet](#) or [Start -> Settings -> Ethernet -> Change adapter settings](#). In other versions of Windows, the navigation to [Change adapter settings](#) will be similar, though maybe not identical. In Windows 10, the user can search for “Ethernet” in the search bar on the bottom of the toolbar.

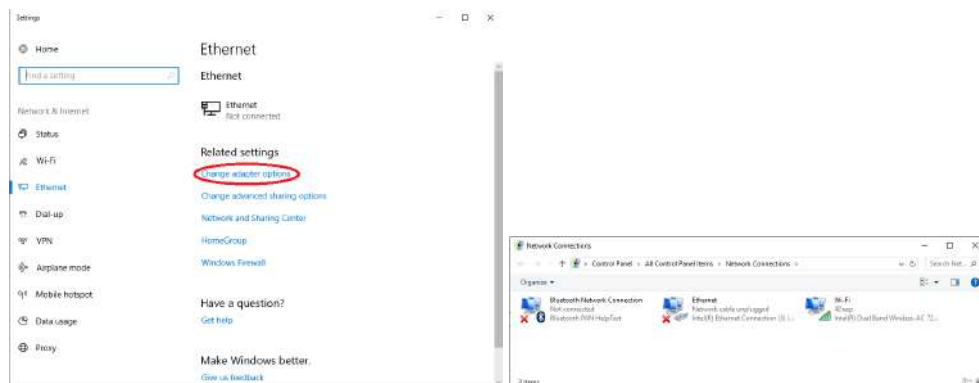


Figure 3: Change adapter options (left). Navigating to the Ethernet settings.

- Right-click on the [Ethernet](#) and select [Properties](#) .

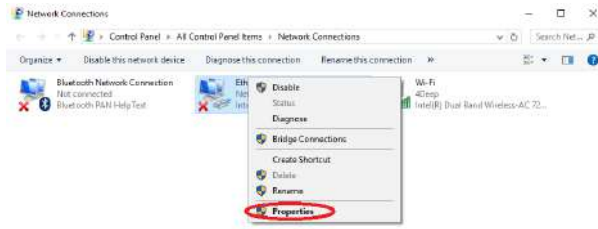


Figure 4: Changing the Ethernet settings.

- Click on **Configure . . .**
- Navigate to the **Advanced** tab. Find **Jumbo Packets** and enable them to the maximum (for example: 9014 bytes).
- Still under the **Advanced** tab, find **Speed and Duplex** from the list and set the value to **Auto negotiation**.
 - Note that if these setting do not work, the **Speed and Duplex** can be set to **1 GBit Full Duplex**.
 - Note that on some Windows OS, the “Speed and Duplex” are may be located under another tab, such as “Link Speed”.

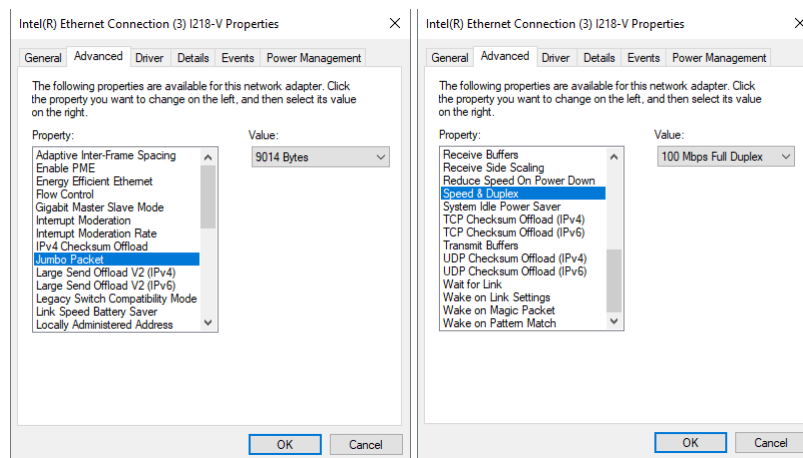


Figure 5: Enabling the Jumbo Packets (left), and Changing the Speed and Duplex (right).

- Click **Ok** and the set up is complete.

2.5 Connection of the HASP dongle and starting the HoloScan software

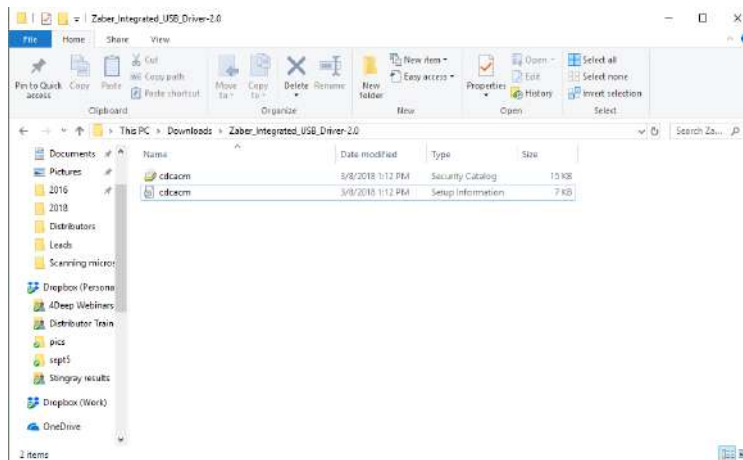
After installation, ensure that the supplied HASP hardware protection key (dongle) is connected to a computer USB port. Make sure the dongle light turns on. Launch the HoloScan software by going into **Windows Start Menu-> 4Deep-> HoloScan**. The software will start. Once it is verified that the software launches, close it for now, as the hardware should be powered on before the software is started.

For information on operating the software, refer to Section 5.

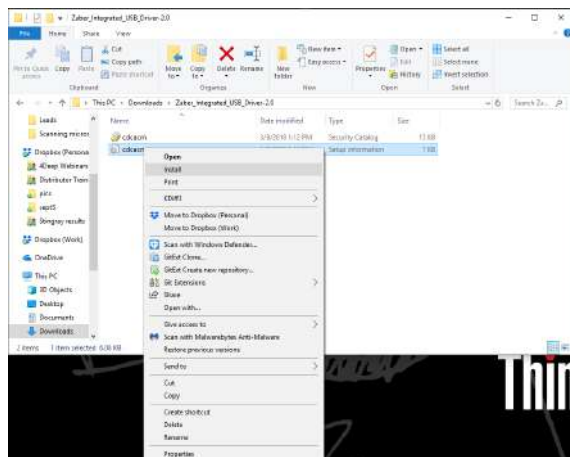
2.6 Download and install the Zaber stage driver

The scanning stage in the HoloScan hardware requires an external driver installation to operate with the HoloScan software.

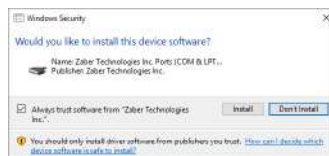
- Go to: <https://www.zaber.com/zaber-software>.
- Find the “A-MCB2, X-MCB USB Driver” and click the Download option.
- Un-zip the folder. There should be 2 files in the folder:



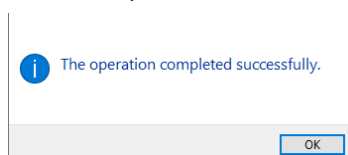
- Right-click on the file with the type “Setup information” and select “Install”.



- Allow Microsoft to install the driver:



- Once completed, the following message should be displayed:



3 HoloScan hardware Installation

3.1 Contents Shipped

Important Be careful when unpacking the contents of the package. As the HoloScan images at the micron/sub-micron level, foreign objects (dust, dirt, fingerprints, smudges, perspiration, saliva etc.) on the camera lens and/or the point source can greatly affect the quality of the captured holograms. Take care not to touch the camera lens and/or the point source. For further Care and Cleaning information, see Section 6.

When the HoloScan hardware is shipped, the entire system will be pre-assembled, except for the power and connection cables.

The following is the list of parts of the HoloScan hardware.

- The HoloScan hardware. NOTE: when the lid of the HoloScan hardware is opened upon delivery, there will be two tie-wraps: one to hold the camera in place, and one to hold the Camera pull bar in the forward position so they are set during shipping. These tie-wraps will need to be removed before the HoloScan hardware is operated.

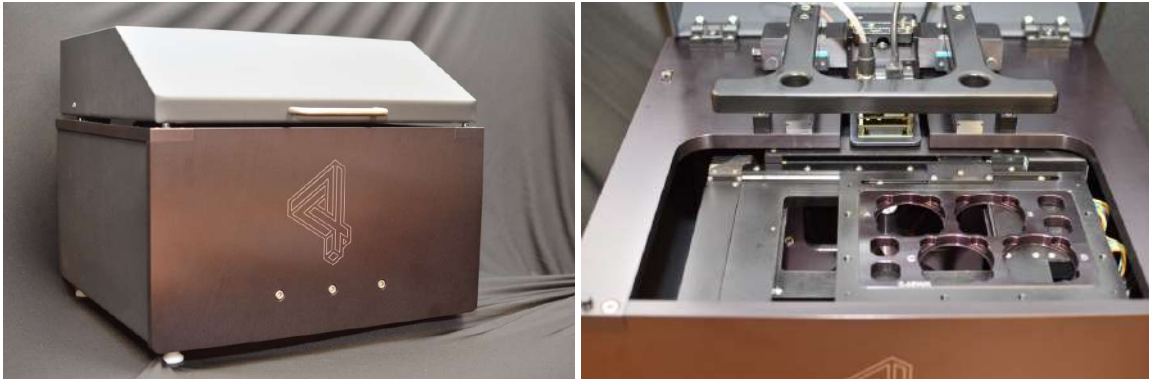


Figure 6: The HoloScan hardware - exterior (left) and interior (right).

- Power supply (12 VDC) for camera system (4 prong). Note that the power connector is keyed.



Figure 7: Power supply for HoloScan hardware (4 prong).

- Power supply (48 VDC) for sample stage (3 prong). Note that the power connector is keyed.



Figure 8: Power supply for sample stage (3 prong).

- USB cable to connect the computer to the sample stage, to control the stage.



Figure 9: Connection cord for sample stage to computer.

- Ethernet cable to connect the HoloScan hardware to the computer for camera connection.



Figure 10: Ethernet cable.

- Sample insets (2). There are 4 Wells in each sample inset, labelled A-D.

Caution Take care when handling the sample insets. The lip on the edge of each Well is very small and cannot be nicked or damaged in any way. If the lip on the Well is damaged, the sample will not be in the correct position to be scanned correctly. If the Sample insets are damaged in any way, contact a 4Deep representative to replace the Sample inset.



Figure 11: Sample inset.

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

3.2 Required Extras (not included)

The sample insets are designed to hold a specific sample dish. These are not included, as most customers have access to these dishes already.

- MaTeK Corporation dish P50G-0-30-F.



Figure 12: MaTek dish with a Resolution target in the dish.

It is recommended that you have 8 or 12 dishes, as 4 can be used in the sample inset.

3.3 Recommended Extras (not included)

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

- Lint-free wipes/swabs (ex: Kim-tech), for use in cleaning (Section 6).
- LED-screen cleaner, for use in cleaning (Section 6).

3.4 The HoloScan hardware and its Components

The HoloScan hardware consists of these primary components:

1. Lid:

- The Lid is simply the lid that covers the microscope.

Caution The Lid must be closed to start and complete a scan. If the Lid is opened during a scan, the entire Well must be scanned again, and the scan cannot be restarted in the same position it was stopped.

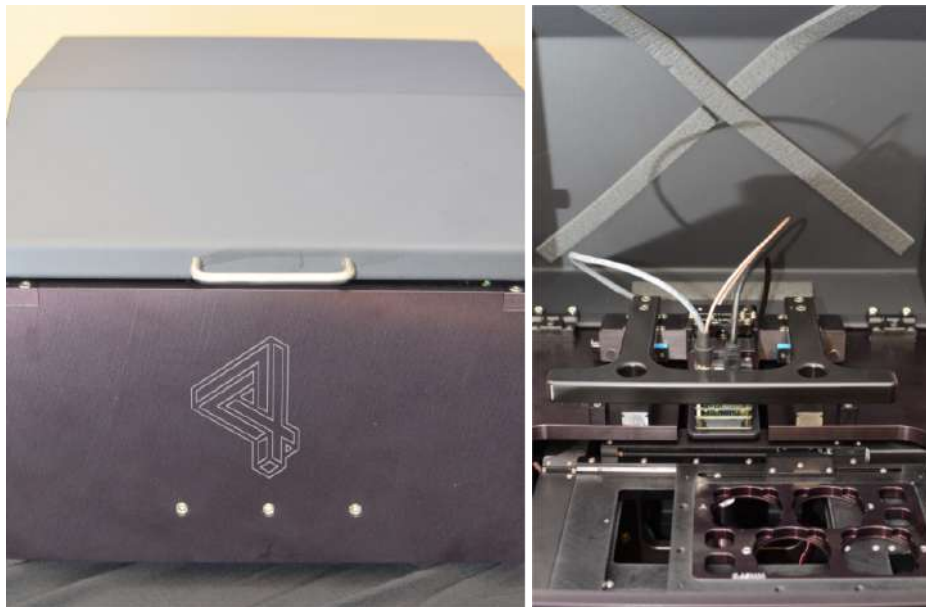


Figure 13: The Lid, closed (left) and open (right). Note that scans can only occur when the Lid is closed.

2. Camera pull bar:

- The camera can be moved forward, towards the sample, and backwards, away from the sample. The Camera pull bar is in the backwards position to allow the user to place the Sample inset into the Sample tray. The Camera pull bar is in the forward position to allow a scan to occur. When the Camera pull bar is in the forward position, the HoloScan software is in its “scanning position”.

Caution If the Camera pull bar is not in the forward position, the HoloScan software will not be able to scan the Wells. Ensure the Camera pull bar is in the forward position before attempting to start a scan.

Caution When operating the Camera pull bar, take care not to slam the bar, in either direction as this can cause mechanical damage/misalignment. Be cautious of the cables in the back of the camera.

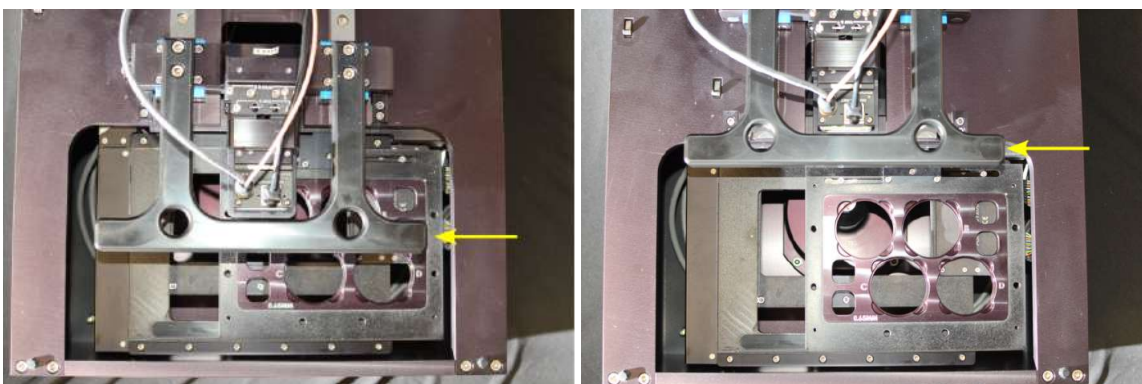


Figure 14: The Camera pull bar in its forward/scanning position (left) and its backward position (right). Note that the Camera pull bar needs to be in the forward position for the scan to start. The yellow arrow in both cases shows where the Camera pull bar is located, and note that this is a top-down view of the HoloScan hardware.

3. Sample tray:

- The Sample tray holds the Sample inset containing the samples. To place or remove the Sample inset from the Sample tray ensure that the Camera pull bar is in its backward position. While it is possible to remove the Sample inset from the Sample tray with the Camera pull bar in the forward position, it is much easier and safer for the hardware to place/remove the Sample inset when the camera is out of the way.
- The Sample tray is outlined in yellow in Figure 15.

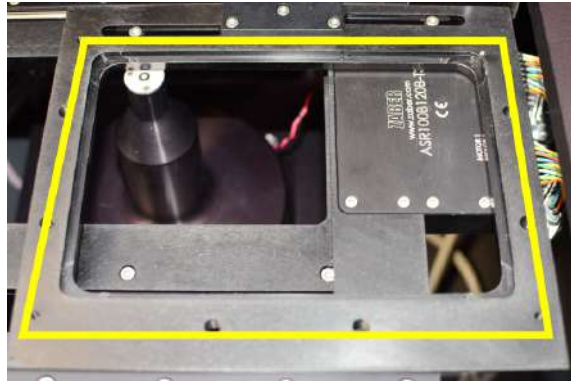


Figure 15: The Sample tray, outlined in yellow.

4. Interlocks:

- There are two Interlocks that need to be engaged for the HoloScan software to start scanning, as mentioned above. As this is a KEY step to set-up the scan, it is repeated to emphasize the importance of having both Interlocks engaged.
- The interlocks are found on the left of the main section of the HoloScan hardware.



Figure 16: The two Interlocks. The left Interlock is to trigger that the Lid is closed, and the right Interlock is to trigger that the Camera pull bar is in the forward position.

5. Back panel:

- Finally, the Back panel contains all of the cable connectors (4) for both power and computer connection, as well as the power switch.
- In Figure 17:
 - Item A is the USB connector for connection from the Sample stage to the computer.
 - Item B is the Ethernet connector for connection from the camera system to the computer.

- Item C is the power connector for the Sample stage (3 prongs).
- Item D is the power connector for the camera system (4 prongs).
- Item E is the power switch

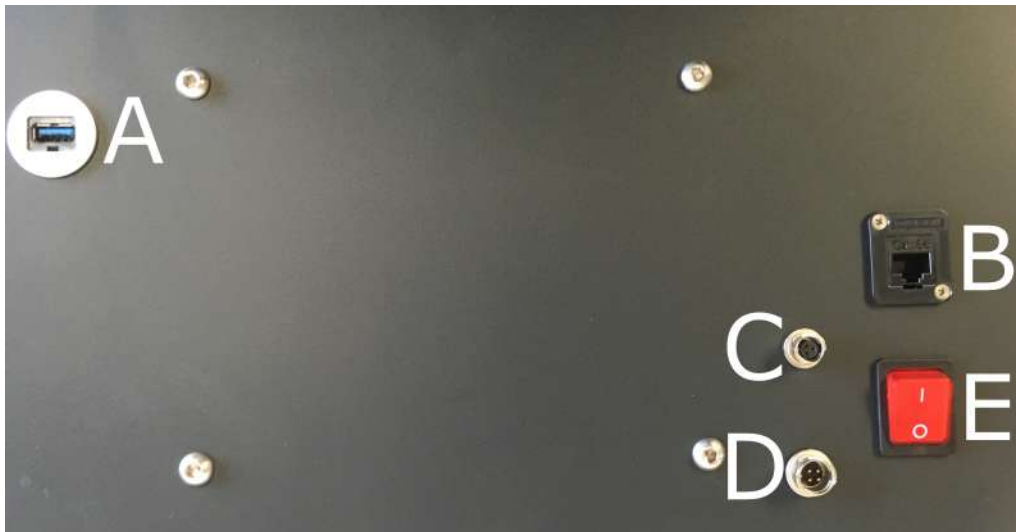


Figure 17: The Back panel.

4 Installation

4.1 Install in a vibration-free environment

The microscope has a high-resolution, thus vibrations will greatly affect the quality of the images collected. Ensure that when the HoloScan is installed, it is installed in a location that is vibration-free (or as close to vibration-free). Note that external vibrations can affect the quality of images collected.

Ensure that when installed, the HoloScan is completely level, again, to ensure the best image quality possible.

4.2 Check the materials shipped

Refer to Section 3.1 to ensure all of the parts of the system were shipped.

4.3 Connect the computer connection cables

- Connect the USB cable from the HoloScan hardware (Item A in Figure 17) from the HoloScan hardware to a USB port in the computer.
- Connect the Ethernet cable from the HoloScan hardware (Item B in Figure 17) from the HoloScan hardware to the Ethernet port in the computer.



Figure 18: Connecting the USB (left) and the Ethernet (right) to the HoloScan.

4.4 Connect the power cables

- Connect the power to the Sample stage using the 3 prong connector (Item C in Figure 17) from the HoloScan hardware to an outlet. Note that the power connector is keyed, thus it must be aligned properly to be powered correctly. The connector needs to be aligned correctly, with the key, then twisted to lock into position.
- Connect the power to the camera system using the 4 prong connector (Item D in Figure 17) from the HoloScan hardware to an outlet. Note that the power connector is keyed, thus it must be aligned properly to be powered correctly. The connector needs to be aligned correctly, with the key, then twisted to lock into position.

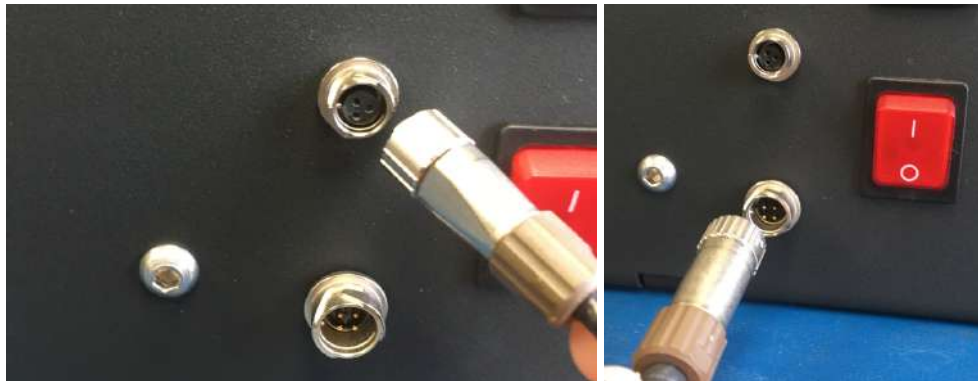


Figure 19: Connecting the camera cables: the power for the scanning stage (left panel), and the power to the HoloScan camera system (right panel).

4.5 Turn the microscope on

- Turn the power on by using the power button on the Back panel of the HoloScan hardware (Item E in Figure 17). You may hear a very light “thunking” noise when the HoloScan hardware receives power.



Figure 20: Turning the HoloScan hardware on.

5 Use the HoloScan system

At this point, the HoloScan software is installed, and ready for use, and the HoloScan hardware is setup and powered on.

5.1 Prepare a sample to scan

As this step will be user-specific, the details are left to the user to determine.

- Prepare the filter/sample so that the filter is transparent.

Caution Holography only works when samples are transparent. If the filters are not transparent, the light from the point source will not be powerful enough to reach the camera. Ensure that the filter is transparent before attempting to scan it.

- The system is currently set up for filters of diameter 25.00 mm. For other sized filters, please contact a 4Deep representative for scanning detail.
- The HoloScan hardware scans up to 4 filters in one run, but can scan less than 4. Determine how many filters you will scan in one run before proceeding.

5.2 Place filters in the HoloScan hardware

Caution Note that in the steps below, steps 3-6 are CRITICAL in collecting good images. The setup of the MaTek dish and the Sample inset need to be placed correctly to ensure the camera is focused on the filter in the MaTek dish, to produce the best images possible. Due to the high resolution of the system, having the MaTek dish or the Sample inset off in its position, but even a few microns, can greatly affect the quality of the images.

1. Open the Lid of the HoloScan hardware.



Figure 21: The Lid is open.

2. Slide the Camera pull bar to the backward position.

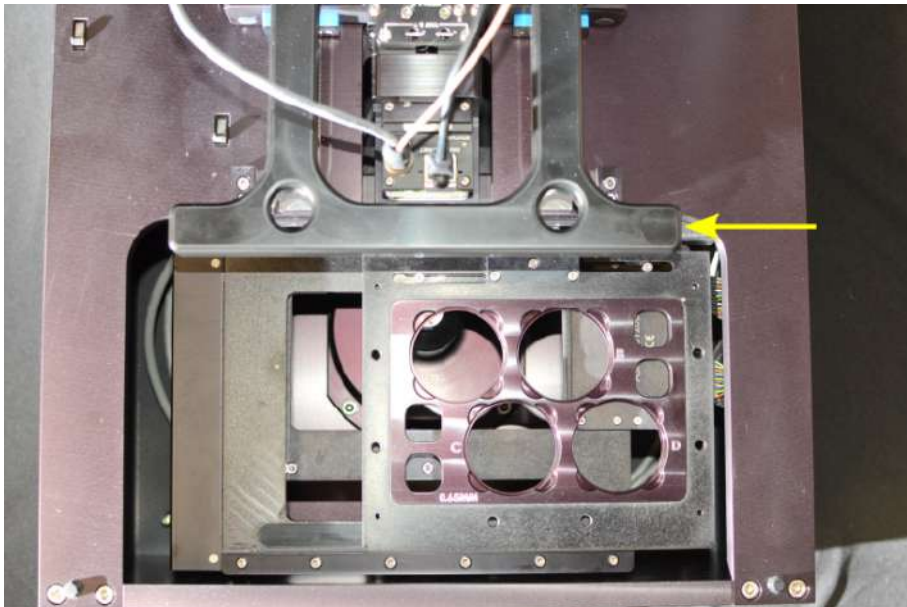


Figure 22: The Camera pull bar in the backwards position.

3. Inspect the Sample inset for any nicks. If the lips of the Well are damaged, the sample will not be in the correct position to be scanned correctly.

Caution If the MaTek dish sits lower in the Sample inset than designed, this can greatly affect the quality of images collected.

4. Inspect the Sample tray for any dust/debris on the lip, where the Sample inset will sit.

Caution If there is dust/debris on the lip, the Sample inset may sit higher than intended. The dish sitting higher may affect the quality of the images collected.

5. Place the filters in the MaTek dish. Place the MaTek dish in the Sample inset.

Caution The MaTek dish needs to sit properly in the inset. If the dish is not placed properly, the resolution of the system will be off, affecting the quality of the images collected. The dish fits very snugly in the inset, thus ensure it is placed correctly.



Figure 23: Placing the MaTek dish in the Sample inset.

6. Place the Sample inset into the Sample tray, ensuring the Camera pull bar in the backward position.

Caution Ensure that the bottom of the Sample inset is clear of any dirt, debris or liquid. The bottom of the inset tray will be close to the point source, which must not be damaged or dirty in any way.

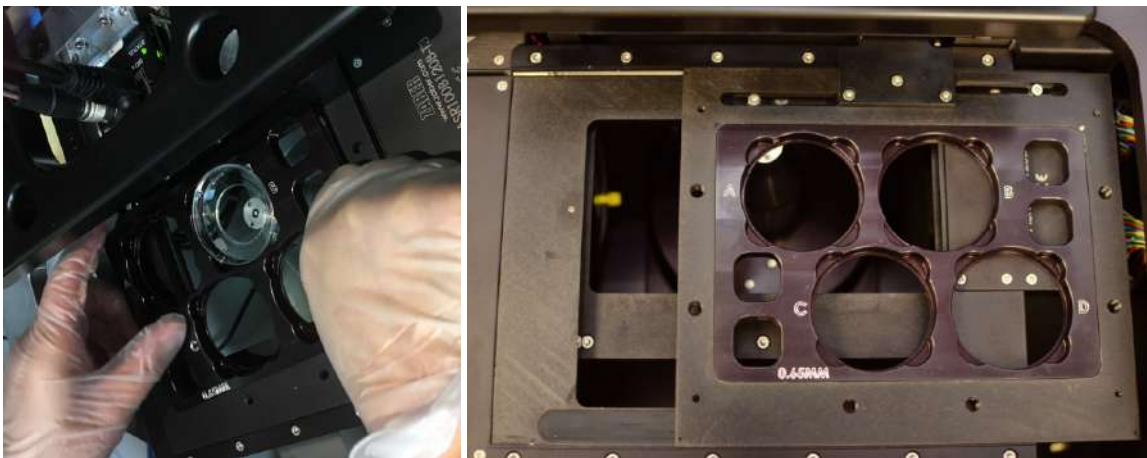


Figure 24: Placing the Sample inset into the Sample tray (left) and once it's been placed (right).

7. Slide the Camera pull bar to the forward position.

Caution The Camera pull bar needs to be in the forward position in order for the scan to start.

Caution When operating the Camera pull bar, take care not to slam the bar, in either direction as this can cause mechanical damage/misalignment. Also be cautious of the cables in the back of the camera.



Figure 25: Moving the Camera pull bar forward.

8. Close the Lid of the HoloScan hardware.

Caution The Lid needs to be closed in order for the scan to start.

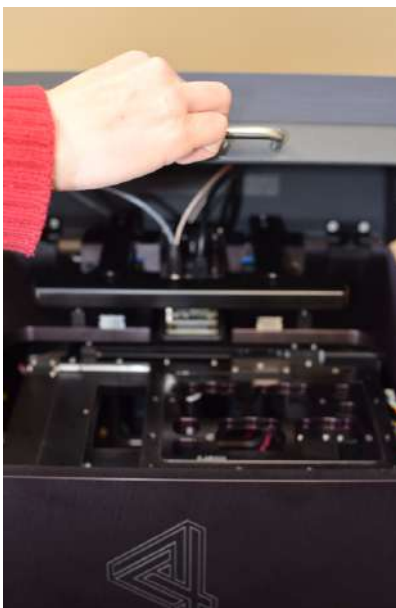


Figure 26: Closing the Lid.

5.3 Launch HoloScan software

Ensuring the HASP key is plugged into the computer, launch the HoloScan software. If everything is connected correctly and powered on, the HoloScan hardware should “home” itself, and you will hear the Sample stage move to its home position.

Caution

If you do not hear the Sample stage move, open the Lid and verify the Camera pull bar is in the forward position. Close the HoloScan software, and close the Lid on the HoloScan. Re-launch the HoloScan software.

- If the HoloScan software does not immediately connect to the camera, you may need to select the camera from a list of connections in a dialog box, as shown below in Figure 5.3. It may take a few seconds for the camera to appear.

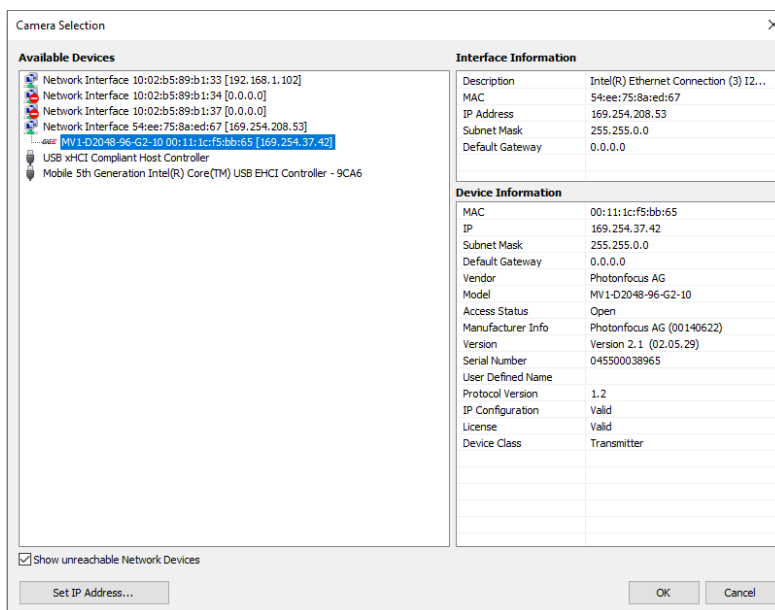


Figure 27: Selection of the camera from the list of possible connections.

5.3.1 The HoloScan software Main Window

Below is the main window of the HoloScan software.

At the top of the screen are the Start and Stop scan buttons, outlined in red in Figure 28.

The large main panel on the left, the Scan display, shows where the scan is currently on the filter. Note there is a tab for each well, to inform the user which well is currently being scanned. The Scan display is outlined in light blue in the Figure 28.

The scanning parameters are shown in the right center panel, shown in Figure 28 in green. See Section 5.4 for more details on the parameters.

The live camera feed is located in the far right panel, outlined in orange in Figure 28.

The bottom left of the screen reports the well currently being scanned and the bottom right shows the progress of the scan; the percent of the well that has been scanned.

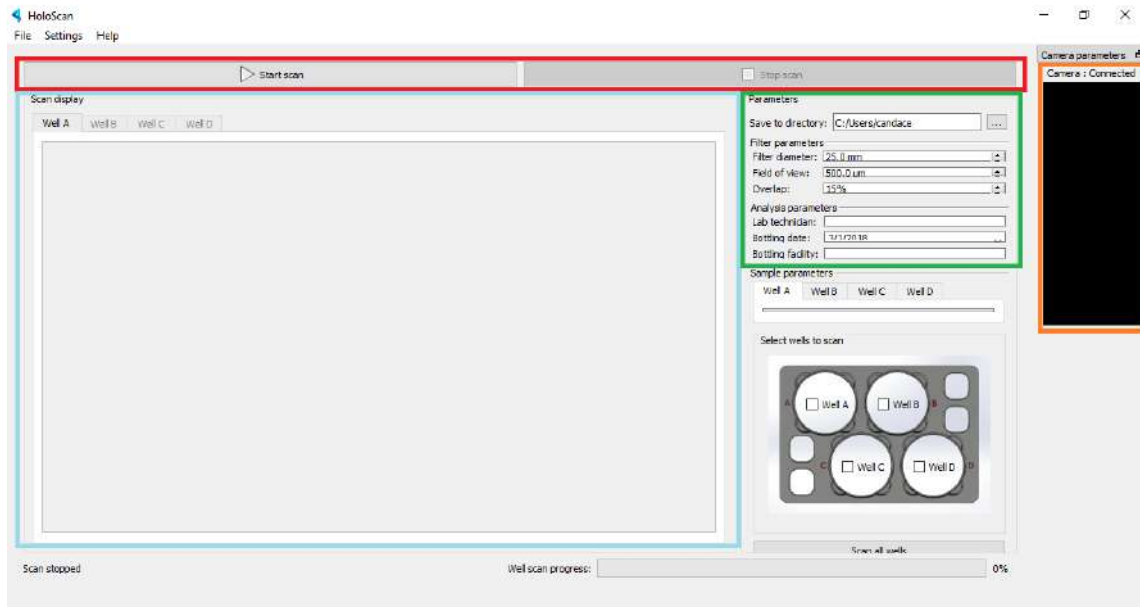


Figure 28: Main screen. Outlined are the features to “Start and Stop scanning”, the “Scanning parameters”, the “Camera feed”, the “Scan display” and the “Scanning progress bar”.

- **File:**
 - Quit
- **Settings:**
 - Camera Options...
- **Help:**
 - About...



Figure 29: Main Menu.

5.4 Setup scanning parameters

- Fill in the scanning parameters:
 - **Save to directory:** select the directory to which the images will be saved (in PNG format).

Caution Ensure that your computer has the space requirements you need before you begin recording so as to not lose important data, as the size of one frame is 4MB. For example, ensure that the selected directory is not the Desktop, as it will fill up quickly.

Filter parameters:

- **Filter diameter:** simply the diameter of the filter. The default value is 25.00 mm, the size that fits into the filter holder.

- **Field of view:** the area that the HoloScan hardware scans in 1 image. The default value is 500 microns.
- **Overlap:** as the edges of holograms are dark, 4Deep recommends imaging an overlap between holograms to ensure the entire filter is scanned. The default value is 10%.

Analysis parameters:

- **Lab technician:** the name of the Lab technician performing the scan. Note this needs to added for the scan to start.
- **Bottling date:** the date the scan takes place. The default is the current date (on the computer).
- **Bottling facility:** the facility the sample is from.

Sample parameters:

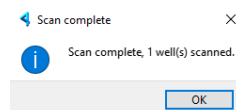
- Once the HoloScan hardware connects to the HoloScan software, the “Sample parameters” for each Well will appear. Note that these parameters are optional and it is left to the user to determine the description of each.

Important The Sample parameters are specific for each Well. Ensure that when using the Sample parameters, they are inputted for each Well separately.

- **Work order number.**
- **Item number.**
- **Blend number.**
- **Wine description.**
- **Packaging size.**
- **Bottling line.**
- **SKU.**
- **Lot serial code.**
- **Blend code.**
- **Select wells to scan:** select the wells that will have filters in to scan. There is also an option to “Scan all wells”, which will select all 4 wells to scan.

5.5 Start the scan

- Simply click the “Start Scan” button. If left to complete, the scan will stop automatically and will report the number of completed scans:



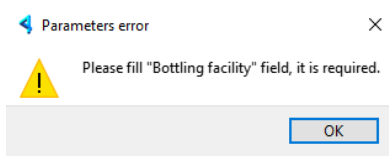
- To stop the scan, click the “Stop Scan” button. Note that if you re-start the scan, it will start the scan from the beginning.

Caution If the scan stops before a Well is fully scanned, the user should navigate to the folder where the images are saved to delete the partially completed scan.

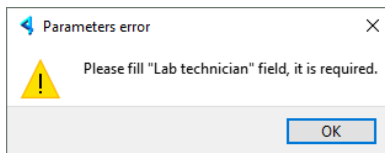
5.6 Possible errors

If not everything is properly set up, there may be error messages that will pop up:

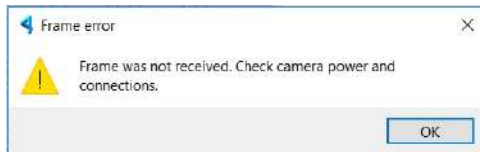
- If the Bottling facility is not inputted, the following error will pop up:



- If the Technician collected the data is not listed, the following error will pop up:



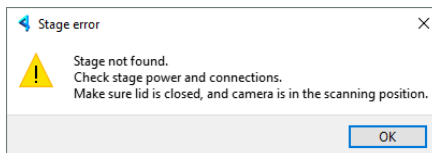
- If the images are not received from the HoloScan, the following error will pop up:



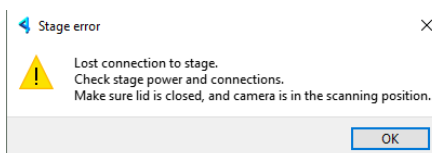
- If the images are not saved to the disk, due to lack of space, the following error will pop up:



- If the stage is not connected or not powered, the following error will pop up:



- If the Lid is opened during scanning, the following error will pop up:



5.7 Post-processing

Each hologram from the scan will be saved in sub-directories under the folder selected in the “Save to directory”. Within the main directory, chosen or named by the user, the HoloScan software will create a folder named from the Bottling Facility inputted above. Within that folder, there will be a sub-directory named for the date. Finally, within this folder, there will be a sub-directory for the time of day.

For example, if the “Save to directory” folder was “Chilean Chardonnay” and the wine was bottled at 4Deep on March 5 2018 at 13:07, the folder structure would look like this:

- C:\Desktop\Chilean Chardonnay
 - 4Deep
 - * 2018_March_05
 - 13 07

The image names contain the Well scanned, the date and time (of the start of the scan), and the position (X and Y in microns) of the image within the filter.

For example, an image from Well D, started at 13:07 on March 5 2018 at position X ->1174 microns and Y -> 2589 microns would be “well_D_05-Mar-2018_13-07_x_1174_y_2589”.

Once all the holograms are saved, they can be processed later in Octopus and/or Stingray software.

6 Care, Cleaning and Maintenance

Your HoloScan hardware requires very little maintenance:

- **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 HoloScan hardware.**

You can clean dust and dirt off the HoloScan hardware 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 when absolutely necessary. The point source can be cleaned dust-free compressed air that is safe for plastics and optics.

Store the HoloScan hardware with the Lid closed to prevent any dust entering the system.

Keep the HoloScan software stored with the power off.

7 Troubleshooting

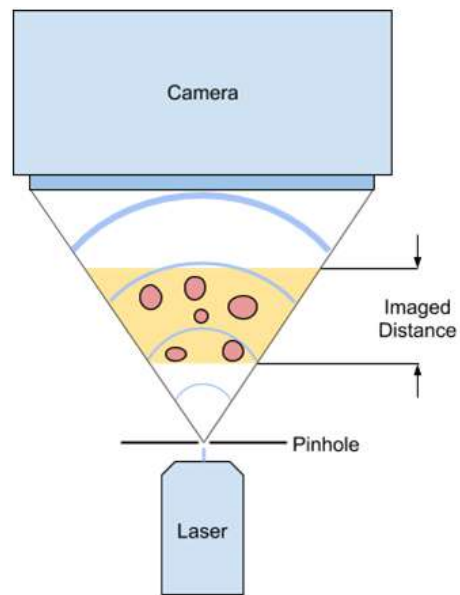
Issue	Solution
HoloScan hardware does not start scan when “Start scan” is selected in HoloScan software.	<ol style="list-style-type: none"> 1. Ensure the Lid is closed. 2. Ensure the Camera pull bar is in the forward position. 3. Ensure all the cables are connected for both power (2) and connection to the computer(2).
HoloScan software does not launch, reporting “Sentinel key not found”.	Ensure the HASP key is connected to the computer.
Cells in holograms are not clear.	<p>The main culprit for this case is that either the MaTek dish or the Sample inset are not in correct position.</p> <p>Ensure the MaTek dish sits flush in the Sample inset. Inspect the Sample inset Wells for nicks on the ledge.</p> <p>Ensure the Sample inset sits flush in the Sample tray. Inspect the Sample tray for dirt or debris, and clean it off, if there is dirt/debris.</p>
HoloScan hardware loses power and does not scan again	If the HoloScan hardware loses power during a scan, both the HoloScan hardware and software need to be restarted. Any images from incomplete scans should be deleted.
HoloScan hardware loses Ethernet connection	If the HoloScan hardware disconnects from the computer during a scan, both the HoloScan hardware and software need to be restarted. Any images from incomplete scans should be deleted.
HoloScan hardware loses connection to stage (USB cable)	If the HoloScan hardware disconnects from the computer during a scan, the scan can simply be restarted with the HoloScan software. Any images from incomplete scans should be deleted.

Table 1: Common troubleshooting problems and solutions

8 Appendix

8.1 Principle of Operation

The HoloScan hardware 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