LISST-HOLO2

User's Manual

Version 1.1

October 2021

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Technical assistance

To obtain technical assistance related to your LISST-Holo2, please contact your local distributor (a list of our international distributors can be found on www.SequoiaSci.com) or Sequoia Scientific, Inc. directly.

Please be sure to include the instrument serial number with any correspondence.

For warranty terms, see www.sequoiasci.com/support/warranty

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1 Introduction to the LISST-Holo2 and In-Line Holography

Thank you for purchasing the LISST-Holo2 Submersible Digital Holographic Camera!

The LISST-Holo2 uses digital in-line holography to obtain in-focus images of particles suspended in water. The holographic method allows all particles within the 50mm long sample volume to be in focus, including details down to 4 microns in size. In-line holography permits only the view of silhouettes (profiles) of individual particles. It permits undisturbed views of the most fragile flocculated particles (flocs), or plankton and other material. The name 'in-line' indicates that a laser beam passing through water is directly imaged on an imaging sensor (e.g. CCD). Thus, the image is made of the overlap of the unscattered laser light and light scattered by particles in the beam. Because this overlap produces interference fringes, the images ('holograms') look like a set of concentric rings or other blurred shapes. The holograms are stored as uncompressed image files.

To see actual particles in the beam, a hologram must be digitally processed, extracting 3-dimensional information from the interference patterns stored in the 2-dimensional hologram.. Reconstruction "slices" the measurement volume into multiple images of planes normal to the laser beam, each focused at a different distance from the imaging sensor. In the LISST-Holo2, the laser beam between glass windows is 5 cm long, and particles are typically reconstructed in 100 planes, each 0.5 mm apart. Thus, from one hologram, 100 in-focus 'photographic' frames are produced, and particles appear in sharp focus at the plane where they existed. The reconstruction software is provided.

Holography is data intensive. Each hologram of the LISST-Holo2 is about 2MB. At the maximum sampling rate of 25 frames per second, that means a data rate of 50MB/second, or 3 GB/minute. However, the LISST-Holo2 operating software gives the user complete control of the frame rate, and also supports sampling in bursts, to adapt to different experimental requirements.

The large number of holograms that might be collected in some experiment requires a method to sort them by importance. Beginning with LISST-Holo2, Sequoia introduced a new algorithm that quickly ranks holograms by the richness of images in them, so that you may see the most interesting ones first, and so on.

2 Overview—Using the LISST-Holo2

This is a very brief overview of LISST-Holo2 operation. For a more detailed introduction to the instrument, proceed to section 3, Getting Started.

Unpack

Open shipping case and remove the LISST-Holo2, white plastic stands, Wi-Fi router, blue 8-pin Ethernet cable, 5-pin Power and Communications Cable and External Power Supply.

Make Connections

- Connect the blue 8-Pin Ethernet cable to the LISST-Holo2 and then a port labeled 1 through 4. Do not use the yellow Internet port. If desired use the yellow Ethernet cable and adapter to extend the length of the blue cable.
- Connect the power to the router and power it up. It will take a couple minutes to boot.
- Connect the 5-pin Power and Communications cable to the 5-pin connector on the LISST-Holo2 which is just below the serial number. This cable will only be used for providing external power. The USB connection is normally not used.
- Connect the External Power Supply to the receptacle in the cable.

Wake the LISST-Holo2

Move the white plastic lever on the Connector endcap from the “0” position to the “1” position and then quickly back to the “0” position. The end cap LED should start flashing yellow, indicating that the LISST is booting. After about a minute, the LED should change to green and flash once every 5 seconds, indicating the LISST is ready.

Connect Wirelessly

Using a WiFi-capable computer or tablet, connect to the wireless network named ***LISST-Holo2*** using a password of ***manyholograms***. Open a web browser and navigate to http://192.168.0.150 . The LISST-Holo2 interface should appear in the browser. (Note that this is the factory-default IP address, and can be changed.)

Set up Sampling

The LISST-Holo2 can be set up with 4 different sampling programs. Pressing the Home tab on the top of the page will display a summary of each sampling program. Click on the hyperlink of the program labeled “Selected” to change its parameters. For testing purposes, select

- Start condition “Magnetic switch is in the 1 position”
- Fixed Rate Mode with a sample interval of 1 second, and
- Stop condition “Magnetic switch is in the 0 position.”
- Click “Save & return to summary”

For more details on sampling programs, see section 5.3.

Collect Holograms

To start sampling, move the white endcap switch to the “1” position. Data collection will continue until you move the switch lever back to the “0” position.

Offload Holograms

The holograms must be offloaded from the internal solid-state drive for processing. Individual Holograms can be offloaded from the Images menu on the LISST-Holo2 User Interface. To offload multiple holograms use an FTP utility like Filezilla (http://filezilla-project.org). Offload the files into a separate directory if batch processing is to be used. For more details, see section 5.7.

Batch Processing of Holograms

Open the Holo Batch program and select File Paths for the Raw Holograms, Reconstructed Images and the Size Distributions. Press the Process button to process all the holograms in the selected folder. For details on Batch processing and installing the Holo Batch program see Sections 4.2 and 4.4

Detailed Processing of Holograms

Run the Holo Detail program to investigate individual holograms in more details than the Holo Batch program. Use the Hologram Content Rating to select a hologram of interest. Select a Region of Interest (ROI) and click on the Reconstruct button. Use the slider to adjust the depth of the reconstructed image. For more information on Holo Detail, see Section 4.5.

3 Getting Started

3.1 Introduction

This section provides simple instructions to quickly learn the operation of the LISST-Holo2. It will walk the user through setting up and capturing hologram within a few minutes after opening the case for the first time. The experienced user may find this section useful for a quick review.

Section 4 describes how to install and use the software for processing the raw holograms. It is not necessary to install any software to go through this section and become familiar with the instrument and its operation.

Section 5 discusses how to configure and deploy the instrument in the field including charging the internal batteries.

3.2 Connect cables and power up

To program and offload data from the LISST-Holo2 you need a PC with a web browser (e.g. Firefox, Chrome, Edge) and access to a power source. The LISST-Holo2 contains internal batteries, however, for this tutorial we'll demonstrate how to use the instrument with external power.

Take out the instrument stands and place the LISST-Holo2 on the stands.

Take out the clear plastic box and open it up.
Inside you will find a few cables and power supplies, described below.

The blue cable is an Ethernet cable used for communication with the LISST-Holo2. The Ethernet cable comes with an attached coupler that allows you to extend the cable by plugging into a second Ethernet cable. The coupler is provided for convenience and is not required for use.

The black cable is a 5-pin cable used to power the LISST-Holo2 when working in the laboratory.

The 5-pin cable carries **power** to the LISST-Holo2 from the included 110/220VAC power supply; it is **NOT** used for communication or battery charging. The USB connector on this cable is for accessing the embedded Linux system, which should only be done under instruction from Sequoia Scientific, Inc.

The blue Ethernet cable must be used for communication.

Look at the LISST-Holo2 connector endcap.

Remove the connector caps and connect the 5-pin cable to the 5-pin bulkhead connector (just below the serial number) and the blue Ethernet cable to 8-pin bulkhead connector (just above the serial number).

Note the white plastic vent cover next to the 5-pin connector, which is used during battery charging.

NEVER OPEN THE VENT EXCEPT AS DESCRIBED IN SECTION 5.2. LEAVING IT OPEN RISKS FLOODING AND DESTROYING THE INSTRUMENT.

The cables are now correctly connected to the LISST-Holo2.

It is necessary to have BOTH cables connected in order to power and communicate with the LISST-Holo2. No power is supplied through the blue Ethernet cable, only communication.
The LISST-Holo2 does have an internal battery but we recommend using external power while operating the instrument on the bench. Charging the internal battery is covered in section 5.2.

A wireless router is provided with the LISST-Holo2 which allows you to connect your LISST-Holo2 to the internet or a local network. Most users will need the router to communicate with the LISST-Holo2. The LISST-Holo2 can be directly connected to a computer, however, this will require custom network settings on your computer. (See **Ethernet Communications** on page 47 for details.)

Connect the blue Ethernet cable to the wireless router. You can either plug the blue wire directly into the router or extend the cable using the provided Ethernet Coupler

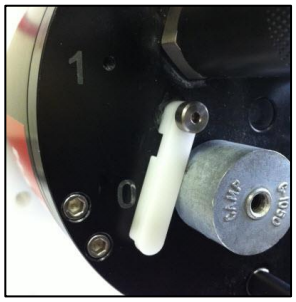
Do not connect your computer or the LISST-Holo2 to the yellow 'Internet' port. Use one of the other four available ports.

Connect the router power cable to the router

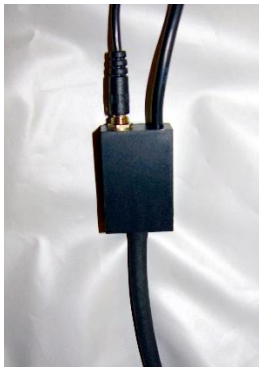
Plug the router power supply into a 110-220VAC power source.

When the router completes its booting procedure, it can be located as **LISST-Holo2** on a wireless network. The password for connecting to the router is **manyholograms** (this information is also located on the router). Establish a Wi-Fi connection between the router and your PC before you proceed.

If you do not have a Wi-Fi enabled PC, you must connect your computer to the router using an Ethernet cable.

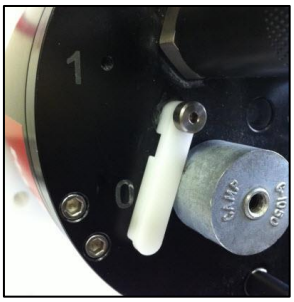


Make sure that the Switch Lever on the connector endcap is in '0' position (off). The Switch Lever does not need to be in the '1' position to communicate with the LISST-Holo2. The Switch Lever will be used to wake the LISST-Holo2.



When you are connected to the router, plug the 19v power supply into the LISST-Holo2 power cable and into 110-220VAC wall power. Supplying power through the 5-pin connector will allow the LISST-Holo2 to operate using external power, without draining the internal batteries.

NOTE: This is not the configuration for charging the batteries. The batteries are charged using the battery charger connected to the 6-pin connector. See section 5.2.



When working with the LISST-Holo2, you will often find the instrument in sleep mode. The instrument enters sleep mode when waiting to sample, when a sampling program has finished, or after 10 minutes of inactivity.

If the endcap LED does not flash green every 5 second, the instrument is in sleep mode.

To wake the LISST-Holo2 from sleep, move the Switch Lever to the '1' position, then back to the '0' position.

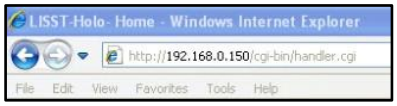
Moving to the '1' position will wake the instrument. Moving quickly back to the '0' position will keep the instrument from starting the current sampling program.

The LISST-Holo2 will boot up. The endcap LED will flash yellow for approximately 40 to 60 seconds. When it is ready, the LED will blink green every 5 seconds. See section 10 for more information about the LED colors and status light indicators.

NOTE: When external power is provided, the LISST-Holo2 will run off external power instead of the internal batteries. If external power is disconnected, the LISST-Holo2 will automatically switch to battery power (assuming the batteries have sufficient charge).

3.3 Take a hologram

If you have followed the instructions in section 3.2 the LISST-Holo2 is now powered up and connected to your PC. It is time to take a hologram!



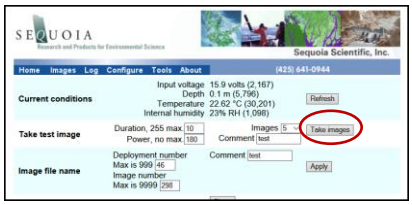
Open up a web-browser on your computer, e.g. Firefox, Google Chrome or Internet Explorer/Edge.

To connect to the LISST-Holo2, enter URL http://192.168.0.150/ in your web browser address bar.

Note: The IP address of the instrument can be changed, and in most cases it must be changed when connecting to a local network – see your local system administrator for more information on how to get a static IP address. See the FAQ in section 9 for more information on how to change the IP address of the LISST-Holo2.

The LISST-Holo2 home page will then show.

To take a hologram, click the **Tools** option.



On the tools page, click the **Take Images** button to take a hologram.

It takes a few seconds to capture a hologram, however, the actual exposure of the hologram is only a few milliseconds.

Then click the Images menu item to go to the Images page, where all holograms on board the memory card are listed.

Click the hologram you wish to view. You will be asked if you want to save it to disk or to view it. Select the Save option, so that you can process the hologram. If you want to process a hologram, you always need to save it to a folder on your PC.

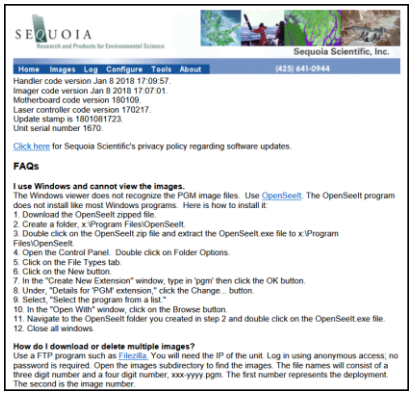
.pgm format

Holograms are stored in .pgm format, which is a lossless image format.

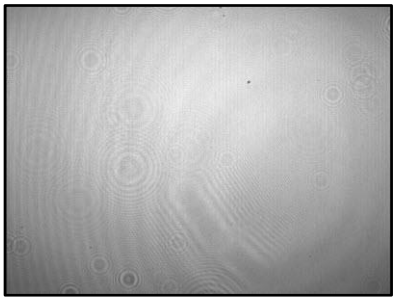
To see the holograms on your PC, you may need to download and install OpenSeeIt (http://openseeit.sourceforge.net/), which is a FREE viewer for pgm files.

See the FAQ on the About page of the web interface for more information about OpenSeeIt.

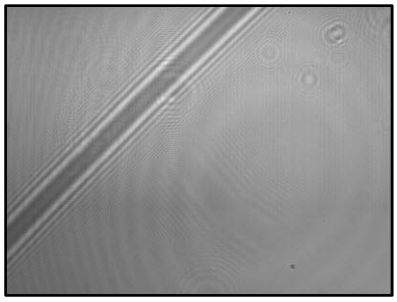
You can of course also use OpenSeeIt to view holograms you have already downloaded and stored on your computer.



The About page shows the FAQ where you can find more information about how to download, install, and use OpenSeeIt to view the unprocessed holograms.



If you took a hologram without anything in the laser beam, your hologram will typically look something like this.

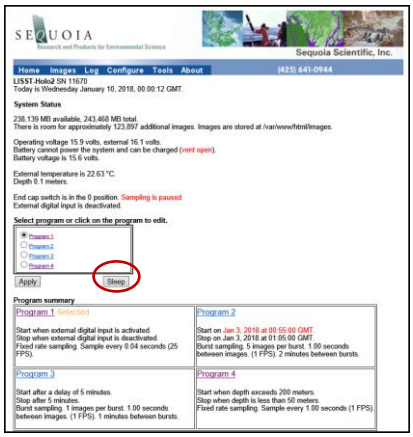


Try taking another hologram, but hold a hair or thin piece of string into the laser beam.

You should then see something like the image to the left.

Take another 1-2 holograms with other types of material in the laser beam, e.g. a screw, or blow some dust through the laser beam when the hologram is captured.

The LISST-Holo2 comes with a test chamber that can be installed between the windows and be filled with water for testing. To install the chamber, remove the U-shaped spacer and press the sliding part into the chamber leaving a small gap. Center the openings of the chamber on the windows and insert the U-shaped spacer so that the O-rings seal on the area around the window. It can be helpful to push the sliding part against the window from inside the chamber before installing the U-shaped spacer.



To put the LISST-Holo2 to sleep, click the **Home** button, then click the Sleep button. If the Sleep button is not present on the **Home** page, go to the **Tools** page and put the instrument to sleep using the Sleep button on the **Tools** page (see section 6.6).

The LISST-Holo2 will also automatically enter sleep mode after 10 minutes of inactivity.

To continue Getting Started with the LISST-Holo2 read Section 4, Hologram Processing with Holo Batch and Holo Detail, or skip to Section 5, LISST-Holo2 Field Deployment.

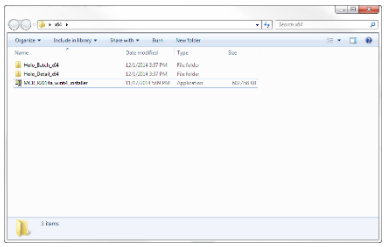
4 Hologram Processing with Holo Batch and Holo Detail

4.1 Intro

The holograms are processed using two MATLAB-based programs called Holo Detail and Holo Batch.

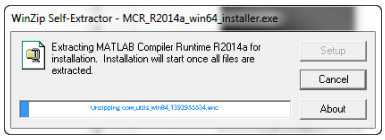
You do not need to be connected to the LISST-Holo2 to process previously offloaded holograms.

4.2 Install the hologram processing software

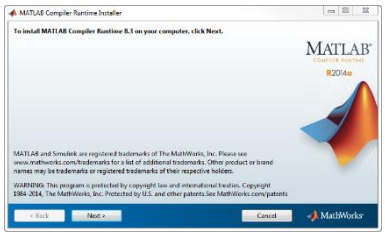


To install the software, plug in the USB memory card that was shipped with your instrument and locate the LISST-Holo2 software folder. Or visit www.sequoiasci.com and download the software package from the LISST-Holo2 page. Our software supports 64-bit Windows and Mac OS X operating systems. You must select the software that matches your machine.

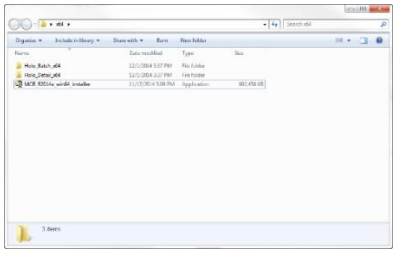
Inside the software folder you will see two folders and one application. The folders contain the Holo Batch and Holo Detail applications. However, before these programs can be run, the Matlab Compiler Runtime (MCR) must be installed. The installer for the MCR is included in the software package and has a name such as 'MCR_R2017a_win64_installer.exe'.



Double click the MCR installer. It will start by extracting files, which may take several minutes. (Note that the installation may appear slightly different on Mac OS X)



After the files are extracted the installer will open. Follow the onscreen instructions to install the MCR. The installation may take a significant amount of time depending on your machine.



The Holo Batch and Holo Detail applications do not need to be installed on your computer. The .exe file contained in each folder can be run as a standalone application. You can move the Holo Batch and Holo Detail folders to a location of your choosing (for example into the Programs folder). It may also be helpful to create a shortcut of the batch and detail applications and place them on the desktop.

Should you like to add the program to the taskbar or start menu, you can right click on the program icon and select 'Pin to Taskbar' or 'Pin to Start Menu.'

4.3 Process holograms using Holo Batch and Holo Detail

Two different programs are supplied for processing holograms; Holo Batch and Holo Detail.

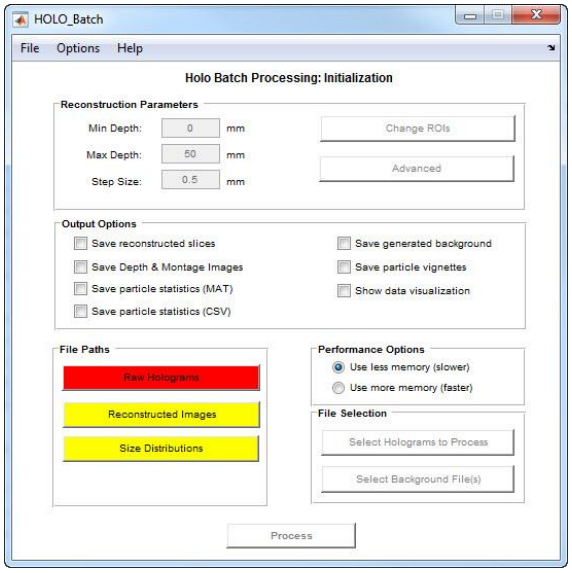
Holo Batch preforms batch processing of holograms, and automatically outputs the size distribution as well as composite images showing the in-focus particles.

Holo Detail is used to sort holograms or perform individual hologram reconstructions. Holo Detail is can be a good way to sort holograms before processing them with Holo Batch.

In the following sections, the use of these two programs will be explained. Videos describing the use of both Holo Batch and Holo Detail are available on the LISST-Holo2 webpage (www.SequoiaSci.com/LISST-Holo2-videos)

4.4 Holo Batch – automated batch processing of multiple holograms

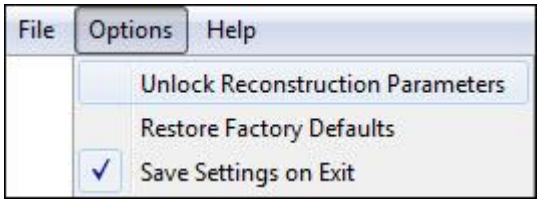
This section describes how to use the automated batch processing program, Holo Batch.



Open the program by clicking the Holo Batch executable.

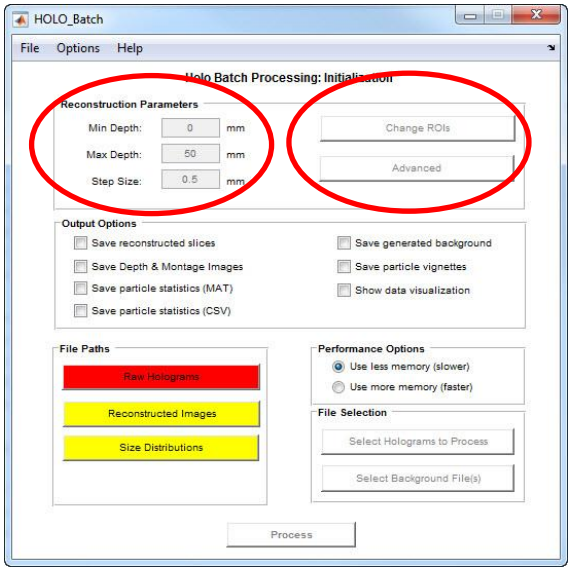
There are 5 main procedures to be performed before processing can begin:

- Select Reconstruction Parameters
- Select Output Options
- Select Particle Bin Size
- Select Paths (Folders)
- Select Files.



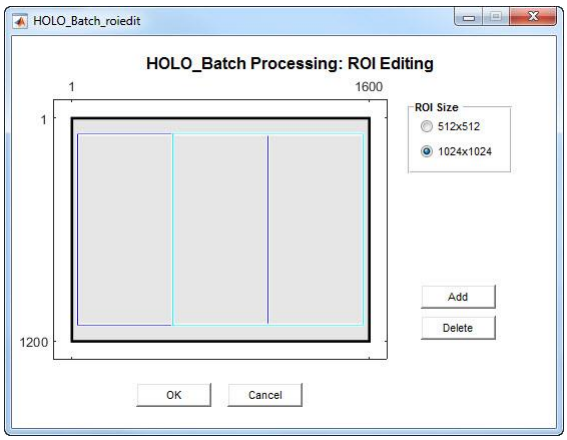
The Holo Batch is shipped with factory default settings for the Reconstruction Parameters, and these should normally not be changed.

However, if necessary they can be changed by selecting Unlock Reconstruction Parameters in the Options Menu.



The Change ROIs and Advanced buttons then become active.

Also, the three input boxes for selection of the minimum and maximum focal length, as well as the step size become active.



The Change ROIs button opens up a window for selecting the ROI (Region Of Interest) to be processed.

The ROI is the part(s) of the hologram that will be reconstructed. Particle counts will only be computed from the reconstructed part of a hologram.

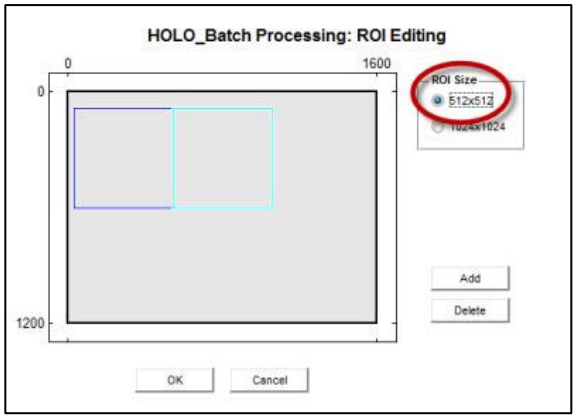
ROI's must be defined as a square region to allow for correct hologram reconstruction. Therefore, the rectangular image (1600 x 1200) cannot be treated as a single ROI.

Once selected, the ROI will be the same for all holograms.

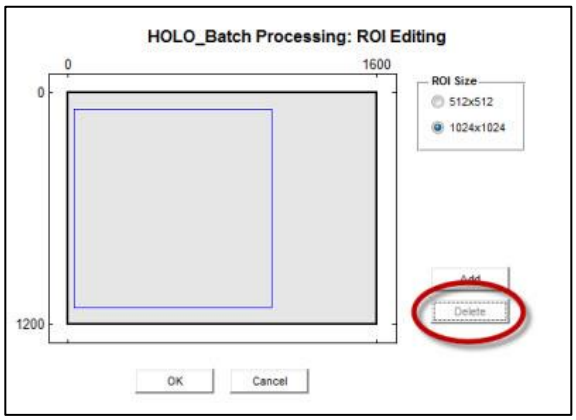
In this example, 2 overlapping ROI's with a size of 1024×1024 pixels are chosen. Particle counts will then be obtained from both ROIs, and adjusted so that particles in the overlapping parts are only counted once.

If you click the Add button, a 3rd ROI will be added, which you can then drag to the desired location.

If you click the Delete button, the last added ROI will be deleted.



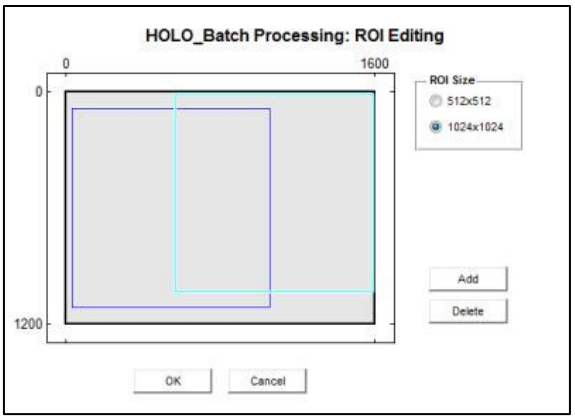
Reconstruction of holograms requires substantial memory, so on PC's with smaller memory (<4GB), or slower processors (<2GHz), the 512×512 pixels ROI should be selected.



Note that you can delete and add ROI's using the Delete and Add buttons.

In this example to the left, one of the 1024×1024 pixel ROI's have been deleted.

Subsequently the Delete button has been grayed out.

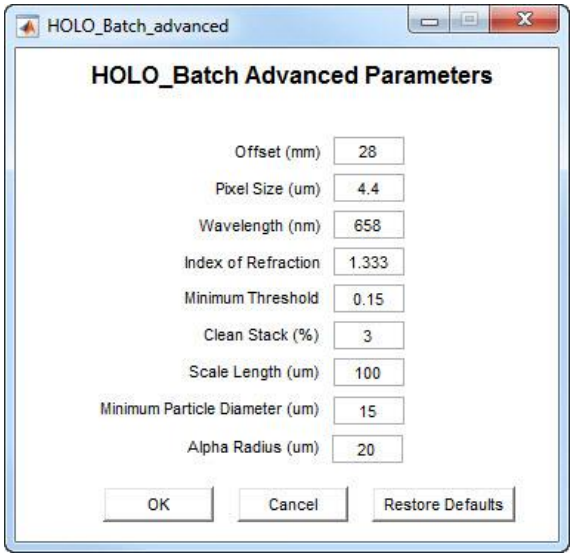


In this example, another 1024×1024 ROI has been added.

Note that the ROIs do not have to be aligned with each other.

Click the OK button when the ROI size and location is satisfying.

Note that the processing time doubles every time you add a new ROI.



The advanced button opens up the settings menu for the advanced parameters.

The values listed in the window to the left are the factory default settings.

With the exception of Minimum Threshold, Scale Length, Clean Stack, and possibly the Index of Refraction, none of these values should ever be changed unless instructed by Sequoia support personnel to do so.

The **Offset** is the distance in mm from the receive window to the CCD array. The reconstruction software needs this distance in order to properly reconstruct the holograms. For the LISST-Holo2 this value is 28 mm. DO NOT CHANGE THIS VALUE.

The **Pixel Size** is the size of each pixel in micro-meters (µm). For the LISST-Holo2 the pixel size is 4.4 µm. DO NOT CHANGE THIS VALUE.

The **wavelength** is the wavelength of the laser in nm. For the LISST-Holo2 the wavelength is 658 nm. The reconstruction software needs this value in order to properly reconstruct the holograms. DO NOT CHANGE THIS VALUE.

The **Index of Refraction** is for the medium in which the LISST-Holo2 is being used. An average value for water is 1.333. If the LISST-Holo2 is used in air, e.g. for measuring pollen or snowflakes, the refractive index should be changed to that for air; 1.0003.

The **Minimum Threshold** is used for creating a composite image of all the particles in the hologram. Decreasing this value will cause more pixels to be identified as being a particle; increasing it will cause less pixels to be identified as a particle.

Clean Stack is used to remove pixels that are less than a certain percentage of the maximum gray scale value. Enter the percentage you would like to be remove here (usually between 0% and 5%). This can be useful for removing background noise.

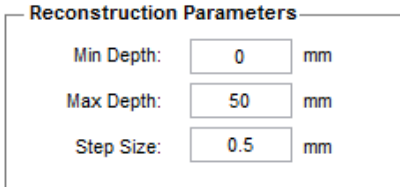
The **Scale Length** is the length of the scale bar (in µm) being added to the composite images created during processing.

The **Minimum Particle Diameter** is a measure used to exclude ‘particles’ that could appear simply due to noise in the image. The default minimum diameter is 15 microns, meaning that unless a particle has a minimum area of at least 9 coherent pixels (Equivalent Spherical Diameter of ~15µm), it is being

excluded from further consideration when computing size distribution etc.

Alpha Radius is the radius of the circle used to produce the alpha shape of a particle. The alpha shape is used to determine the area of particle. More information on alpha shapes can be found here: http://doc.cgal.org/latest/Alpha_shapes_2/index.html.

Click OK when you are satisfied with your selections.

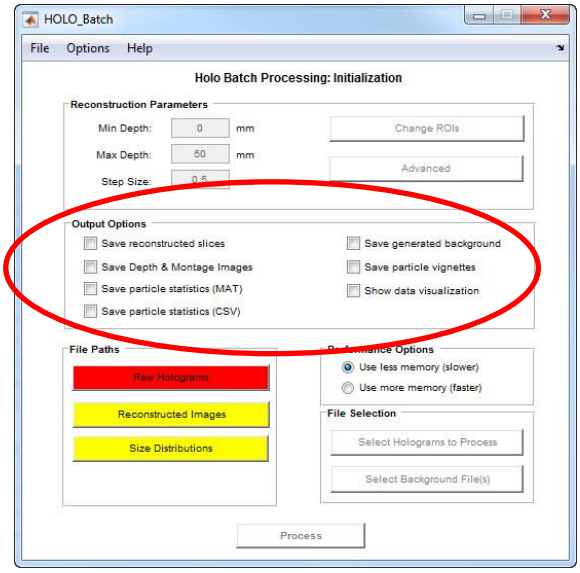


If you wish, you can change the Reconstruction Parameters.

Min Depth indicates where to start reconstructing images. A value of 0 mm will start reconstruction at the receiving window of the LISST-Holo2. **Max Depth** indicates where to stop reconstruction. A value of 50mm will stop the reconstruction at the opposite window (transmit window) of the LISST-Holo2. **Step Size** determines how many millimeters apart the reconstructed images will be.

In the example to the left, the entire sampling volume will be reconstructed (0-50mm), at an interval of 0.5mm. This will result in 101 reconstructed images.

Note that the processing time increases linearly with the number of Steps. Upon processing, all particles in all intervals (slices) will be combined into one focused image.



The next step is to select output options.

A csv file containing the size distribution for each hologram is written by default, even if none of the check boxes or the right are selected.

During hologram reconstruction, an image containing both in focus and out of focus particles is created at every step. Check ‘Save reconstructed slices’ if all slices are to be saved.

‘Save Dep/Mon Images’ saves depth and montage images of particles. These are composite images that show all the detected particles in one 2D plane. Examples are show on page 32.

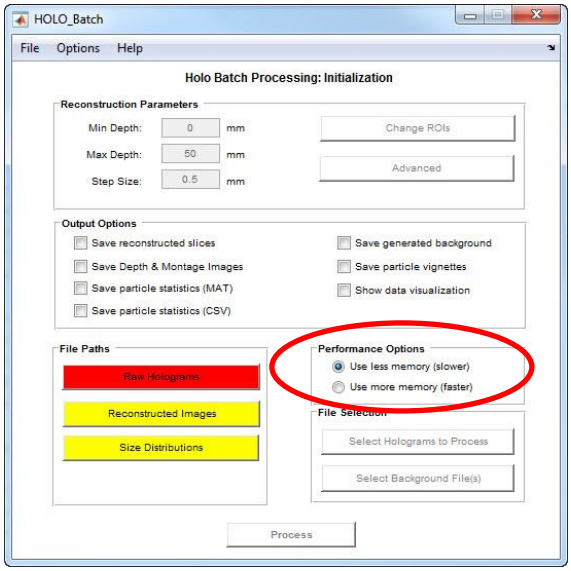
The ‘Save particle statistics (MAT)’ option creates a particle statistics file in MATLAB .mat format for every image processed. It will contain information about each particle detected in the image. See page 24 for details.

The ‘Save particle statistics (CSV)’ option creates a particle statistics file in Comma-separated values (ASCII) format for every image processed. It will contain the bulk properties of the image. See page 23 for details.

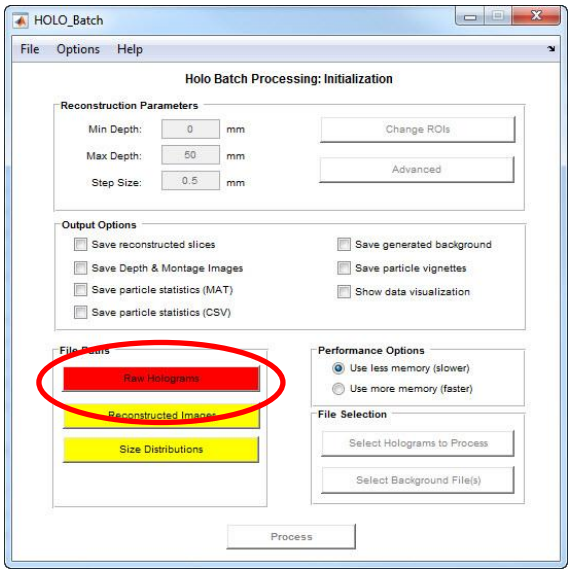
The ‘Save generated background’ option saves the background image used for processing **if** background files are selected (background removal is discussed later in this section).

‘Save particle vignettes’ will save a small image of each individual particle.

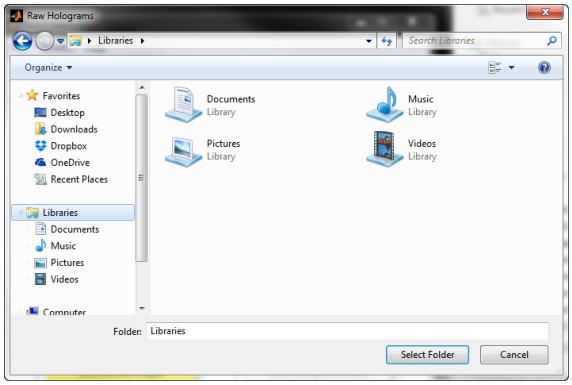
‘Show data visualization’ will show continually updated plots of total volume concentration, number of particles per hologram, temperature, and depth. This option is the fastest way to visualize data after it has been collected.



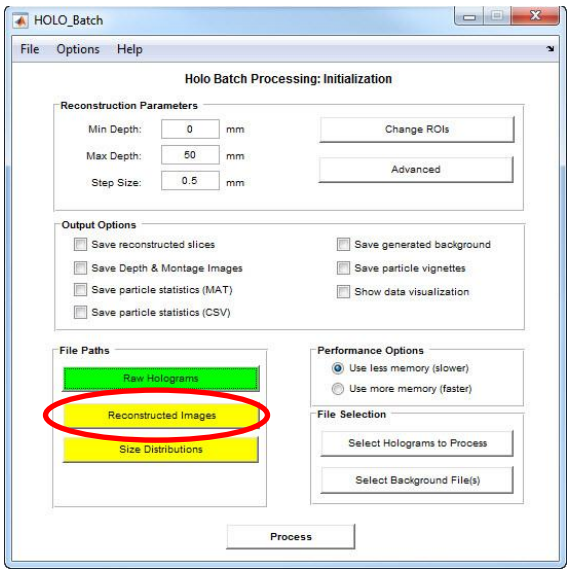
Next, select Performance Options. The default selection is for 'Use less memory (slower)'. The 'Use more memory (faster)' option should not be used unless you are running a computer with at least 6 GB of RAM and are using a reconstruction step size of 0.5 mm or more. If this option is selected a warning message will remind you of the requirements of running the higher memory setting.



Now click the Raw Holograms button in order to select the path from where Holo Batch will read your raw holograms.

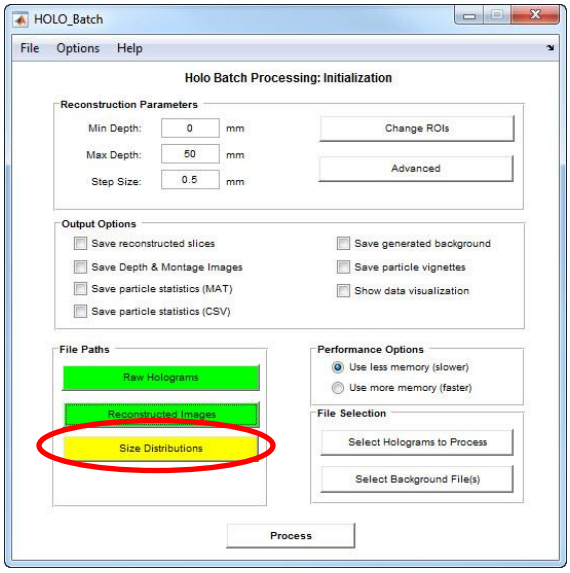


Select the folder where your raw holograms are stored and click OK.



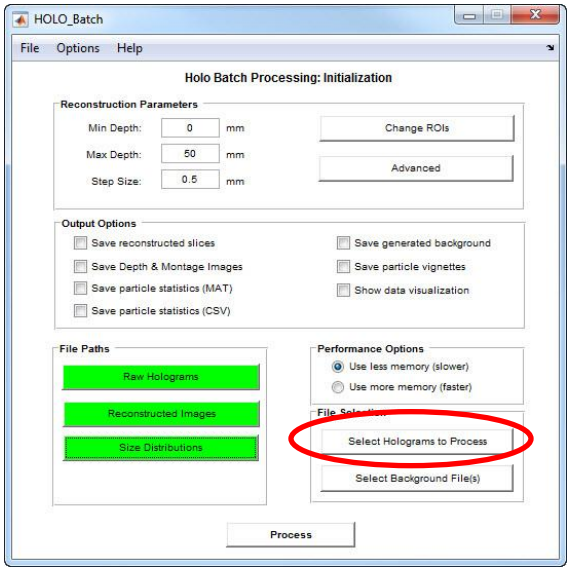
Now click to select the folder where you want the reconstructed images to be stored. This is where reconstructed and Dep/Mon images will be stored. If these options are not selected in the output options, nothing will be written to the folder you select.

As above, a browser window will open up prompting you to select a folder.



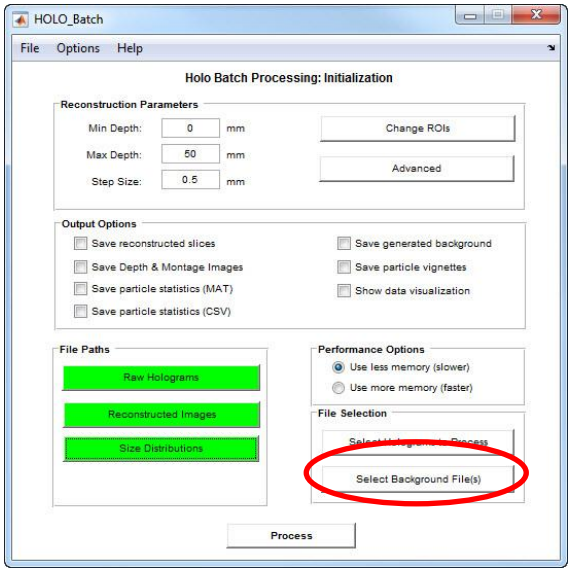
Finally, click the Size Distributions button to select the folder where you want the particle size distributions to be stored.

If you have selected to output .CSV or .MAT files, this is where they will be stored.



If you would like to process a subset of holograms in the directory, you can press ‘Select Holograms to Process.’ The directory you have selected for ‘Raw Holograms’ will open in a browser window, you can now select which holograms you would like to process.

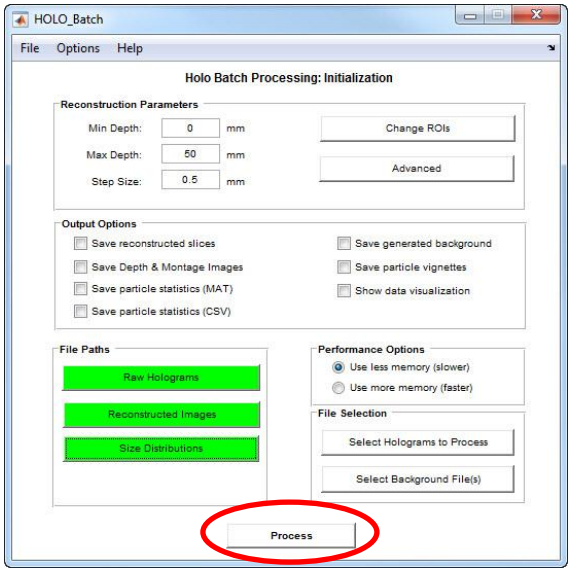
If you would like to process all of the holograms in the directory, you can ignore this button.



If you have a hologram obtained in clean water or air, that you would like to subtract from the holograms, select it here.

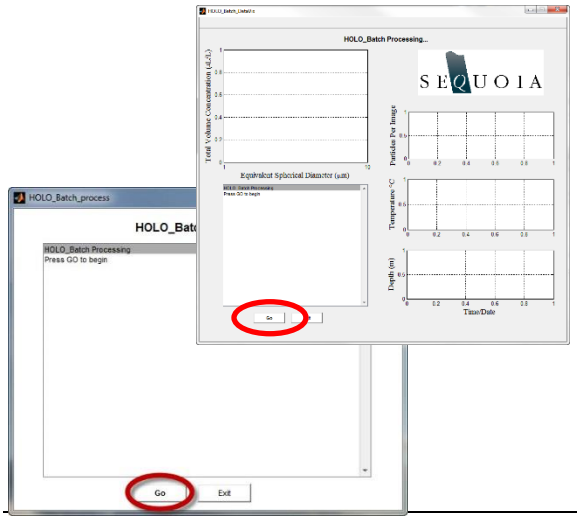
Selecting a background hologram can reduce the noise in the composite output image. You can select multiple background files, which will be averaged into one background file before subtracting.

It is not necessary to supply a background hologram in order to process your holograms; if you do not select a background none will be subtracted.



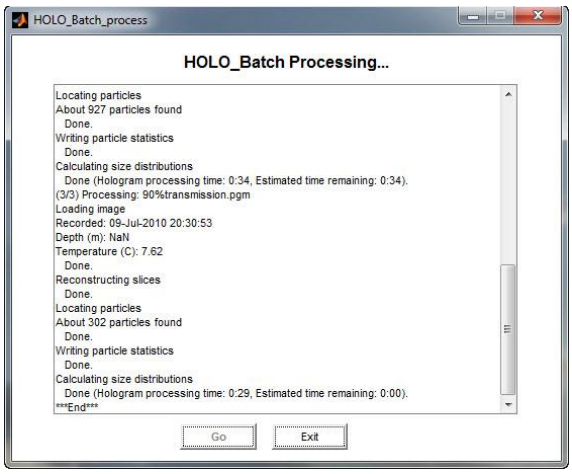
All selections are now complete!

Click the Process button to process the selected holograms.



One of the two screens on the left will appear, depending on if you chose to show data visualization or not.

Press the ‘Go’ Button to start the processing.

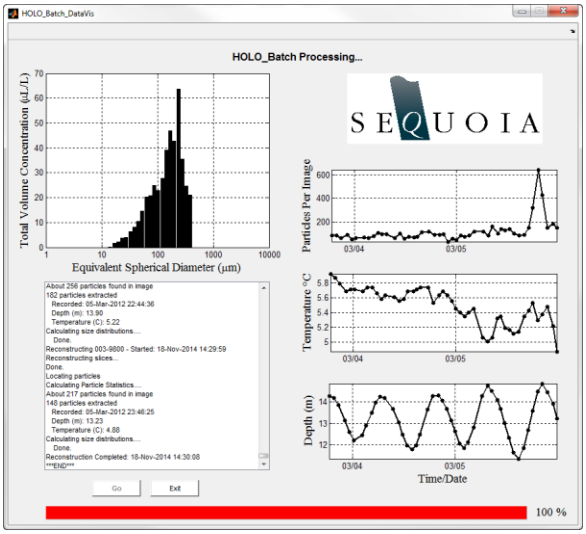


Processing updates will be printed to the text box in the processing window.

Processing times per hologram vary greatly. The time depends on: the size and number of ROIs, the reconstruction step size, selected outputs, performance option selection, number of particles, and computer hardware.

Processing is complete when ‘End’ is displayed in the text box and the ‘Cancel’ button changes to an ‘Exit’ button.

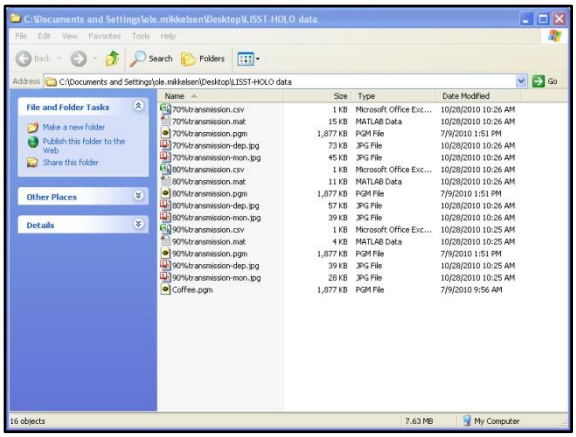
Processing can be aborted at any time by pressing the ‘Cancel’ button. It may take a minute or so for the processing to exit.



If the data visualization option is selected, the plots shown at the left will be updated after each hologram is processed.

A status bar will be displayed at the bottom of the screen showing the progress of the batch processing.

After processing 100 holograms the plots will begin to scroll with the data, this avoids using an excess amount of memory for visualization (i.e. the plots only show the last 100 holograms worth of data).

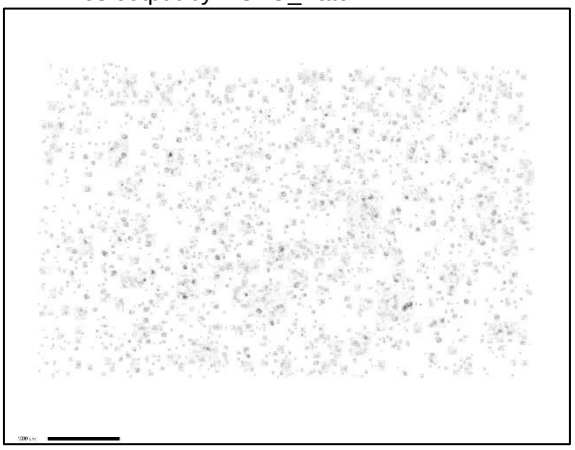


Go to the folder(s) you specified as output paths for your processed data.

You will notice that several files have been created for each hologram. The exact number of files created depends on what you have selected for output options.

These files are briefly described in the following sections.

.TIFF files output by HOLO_Batch

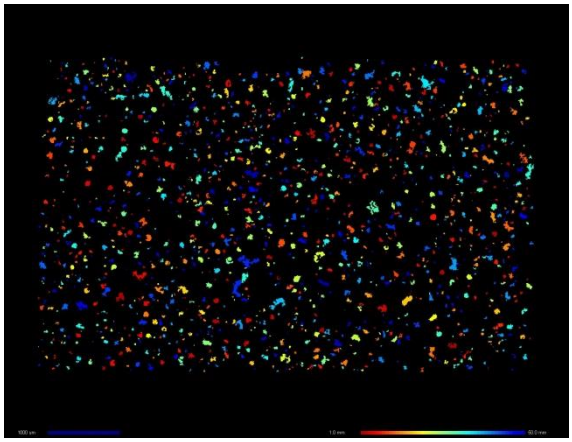


If you selected to output dep/mon images, two .TIFF files will be created for every hologram process. They will be placed in the reconstructed images folder

The –mon suffix image is a montage of all the in-focus particles that were found in all the planes within an ROI.

The scale bar length can be adjusted prior to processing in the Advanced section of Holo Batch.

Note that only the part of the hologram covered by the ROI is displayed on the –mon image. The rest of the hologram was not analyzed and therefore cannot be displayed.



The –dep suffix image is a color coded image, where the particles within the ROI have been colored according to their depth of focus.

As with the –mon image, only the part of the hologram covered by the ROI is displayed on the –dep image.

The scale bar is all blue and displayed in the bottom left corner. The color bar in the bottom right corner shows the location of the particles relative to the transmit window. Red particles are very close to the transmit window, blue particles very close to the receive window.

Using a pair of red and blue 3-D glasses will display the particles with a 3-D effect, where the red particles will appear closer to the viewer than the blue particles.

.CSV files output by HOLO_Batch

A composite file with the name *firsthologram_lasthologram_*All.csv, where firsthologram and lasthologram are the filenames of the first and last holograms that were selected for processing. This file is always generated for each run of Holo Batch. This composite file has all the information about sample date and time, water depth, temperature, and the size distribution (given as the volume concentration in (µl / l) per size bin).

Finally, the composite file has in its first row the lower limit of the size bin for the size distributions in columns 26:75. In the 2nd row is the mid-point of the size bin for the size distributions in columns 26:75 and in the 3rd row is the upper limit of the size bin for the size distributions. The table below shows the parameters in columns 1-75 of the .CSV file.

Column #	***Value***	***Unit***
1	Year (YYYY)	N/A
2	Month (MM)	N/A
3	Day (DD)	N/A
4	Hour (HH)	N/A
5	Minute (MM)	N/A
6	Second (SS)	N/A
7	1/100s of a second (hhh)	NOTE: Currently not available, NaN (Not a Number) displayed

8	Depth	m
9	Temperature	°C
10	Input Voltage	V
11	Exposure	N/A
12	Laser Power	N/A
13	Laser Photo Diode	N/A
14	Brightness	N/A
15	Shutter	N/A
16	Gain	N/A
17	Deployment ID	N/A
18	Image Number	N/A
19	Number of Particles	N/A
20-24	Reserved for Future Use	N/A
25	Total Volume	ul / l
26-75	Size Distribution	ul / l

.MAT file output by Holo Batch

If the 'Save particle statistics (MAT)' checkbox was selected, a MATLAB .MAT file will be created. The .MAT file can only be opened up in MATLAB. It contains a structure called PartStats. It has dimensions N×17, where N is the number of particles detected in the ROI.

The 10 columns have the following information about each particle:

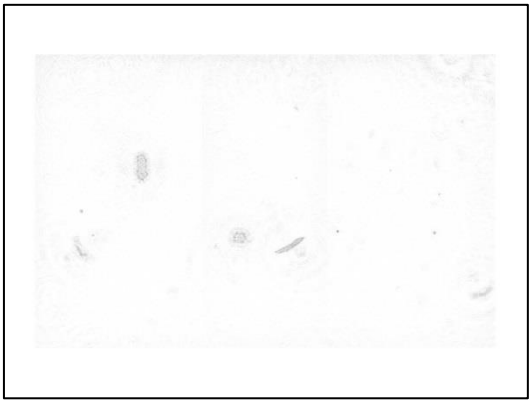
Column #	***Particle Property***	***Unit***
1	Particle Area based on pixel count	μm^2
2	Particle Equivalent Diameter based on pixel count	µm
3	Major Axis Length	µm
4	Minor Axis Length	µm
5	Solidity	Dimensionless
6	Eccentricity	Dimensionless
7	Filled Area	μm^2
8	Convex Area[1]	μm^2
9	Equivalent Convex Area Diameter	µm

[1] The Convex Area is the area, based on pixel count, of all pixels within the convex hull of the particle. The convex hull is the smallest polygon that can contain the particle.

10	Particle Volume	μm^3
11	Centroid (X,Y) (from top left of hologram)[2]	Pixels
12	Focus Depth (from emit window)	mm
13	Bounding Box	Pixels
14	Orientation	Degrees
15	Euler Number	Dimensionless
16	Extent	Dimensionless
17	Perimeter	µm

.TIFF files of individual slices

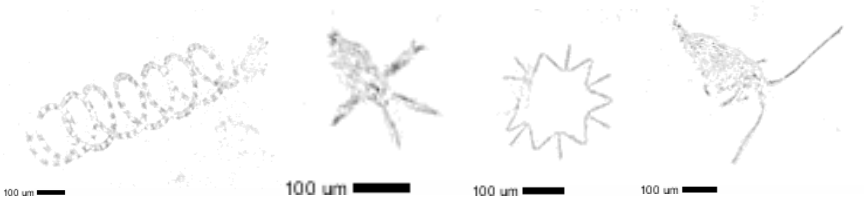
If the check box 'Save Individual Slices' was checked, Holo Batch will save a .TIFF image for each reconstructed slice, showing the in focus and out of focus particles that appear on that particular slice.



The distance from the transmit window of each slice is given in the image filename. For example, the image on the left is 016-0270-z22.500.TIFF, meaning it was reconstructed from hologram 016-0270.pgm at a distance of 22.5 mm from the transmit window.

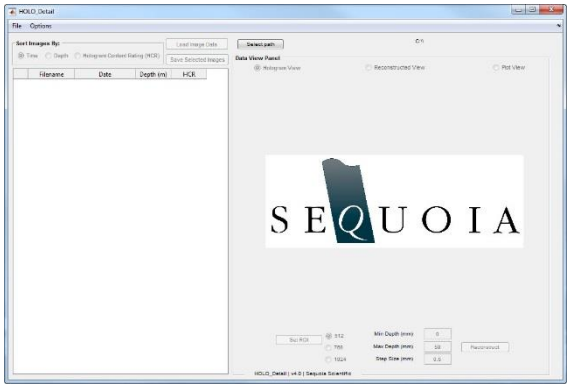
Particle Vignettes

If you selected to save particle vignettes, a small .tiff image of each particle will be saved in the reconstructed images folder.



[2] The Centroid X, Y locations specify where the particle is located. Together with the Focus Depth, these 3 numbers locate the particle in the 3-D space of the laser beam.

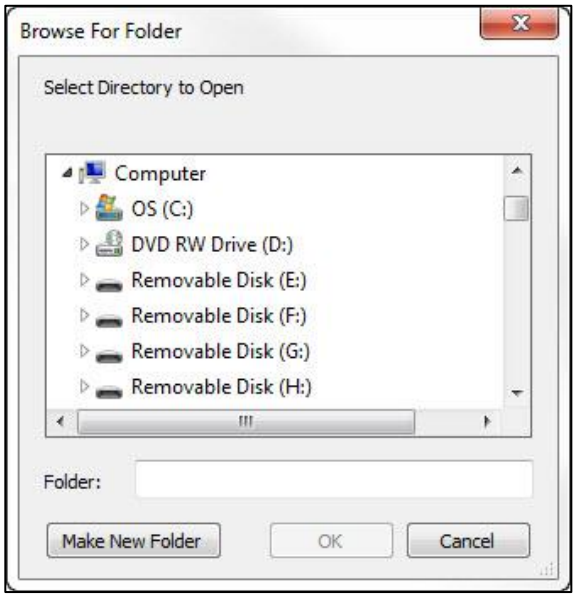
4.5 Holo Detail – processing of individual holograms



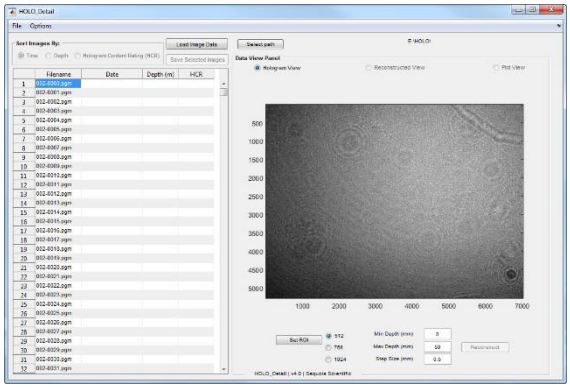
Open the program by clicking the Holo Detail executable.

Select Path

Click the **Select Path** button to select a folder for browsing your holograms.



Select the folder where your holograms are stored, then click OK. If you are just getting stated, navigate to the ‘Example Holograms’ folder located on the ship disk.

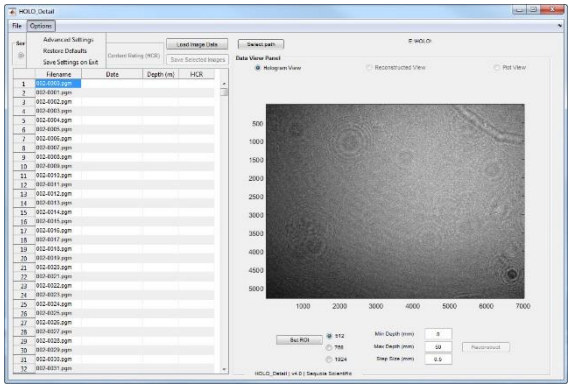


Select a hologram from the list on left.

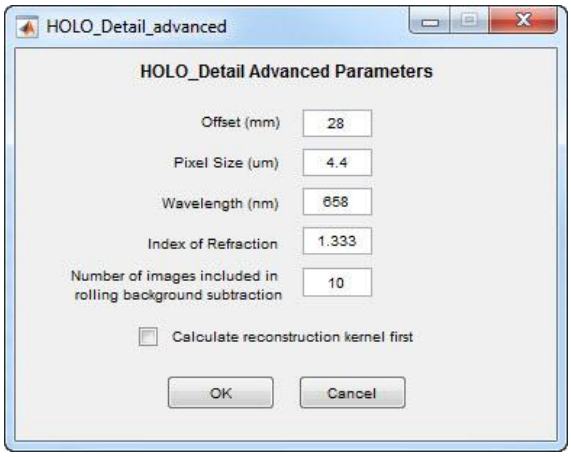
The selected hologram will then be displayed.

Only one hologram at the time can be selected, displayed, and processed in Holo Detail.

Advanced Settings



Additional reconstruction options are available under Options -> Advanced Settings.

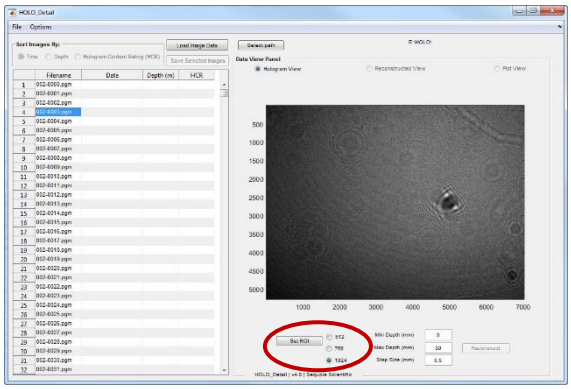


The only parameters that should be adjusted are the index of refraction and number of images in rolling background subtraction. An average value of 1.333 for water is set as the default. This should be changed if the hologram was taken in air or in any other substance with a different index of refraction.

The number of images included in rolling background subtraction is the number of images to average together and subtract from a hologram when computing the Hologram Content Rating (discussed later). This is a rolling background subtraction. For example, the default value is 10, therefore, the holograms averaged together would be +/- 5 holograms from the current hologram.

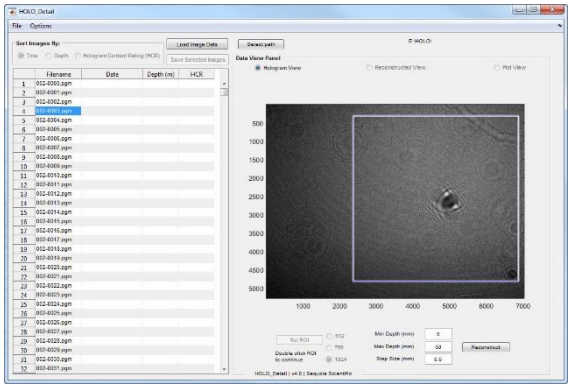
The Offset, Pixel Size, and Wavelength are fixed for the instrument and should not be adjusted.

Set ROI and Reconstruct Buttons

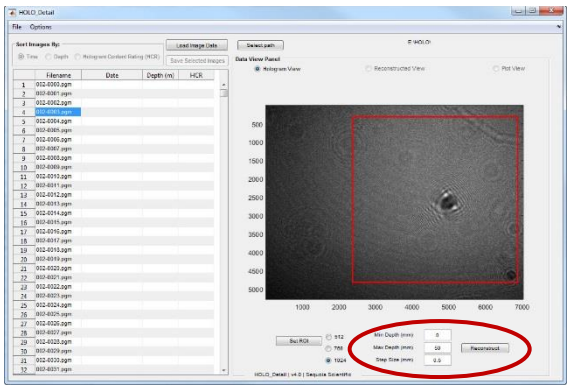


Select the size of the ROI using the radio buttons below the hologram.

After selecting the ROI size press the ‘Set ROI’ button.



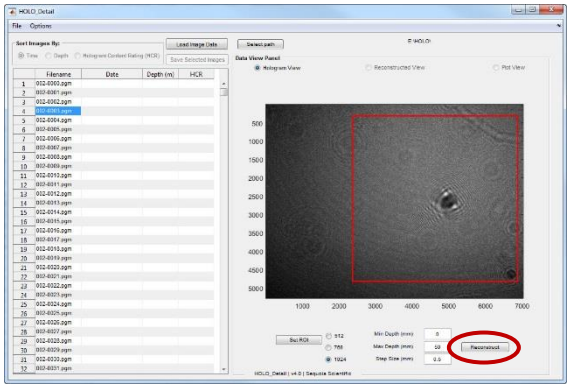
The ROI box will appear as a blue square overlaid on the hologram. The ROI can be moved to another location by moving the mouse over the ROI, selecting it, and then moving it around to the desired location within the hologram.



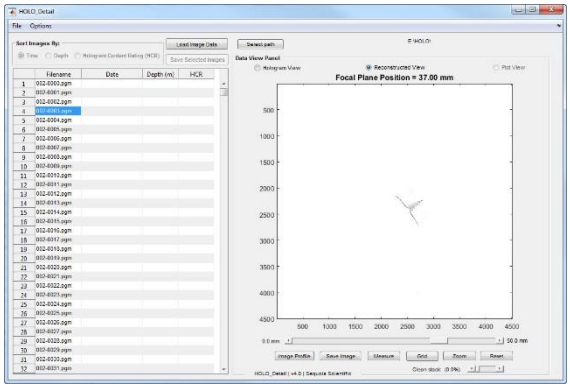
When the ROI is in the desired location, DOUBLE-CLICK it so that the ROI turns red.

Note that the **Reconstruct** button then becomes active.

Now set the minimum and maximum depth for the hologram reconstruction, as well as the step size (in mm).



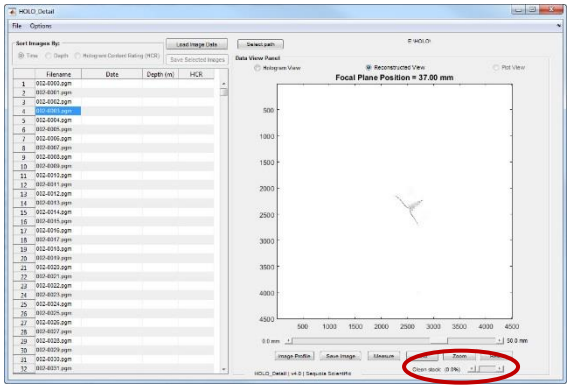
Click the **Reconstruct** button to initiate the reconstruction process.



Upon reconstruction, the individual slices can be viewed on the right by clicking on the left and right arrows.

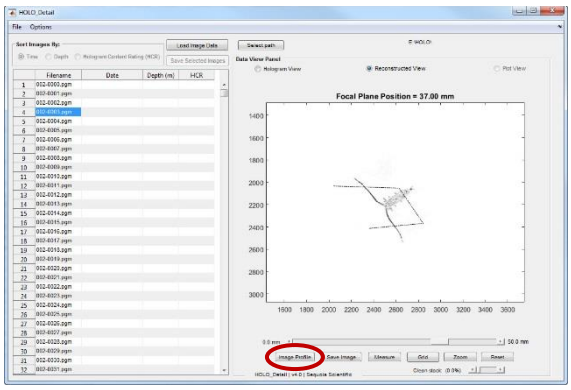
The X, Y, and Z axes of the reconstructed image are in calibrated units (µm). The Z axis (the focus depth) is displayed above the reconstructed image.

Clean Stack Slider



The Clean Stack slider is used to reduce noise in the image. The slider can be moved from left to right to help remove background noise in the images.

Improfile Button



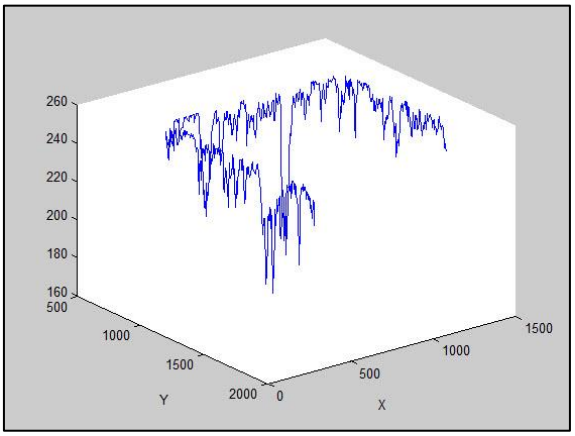
The **Image Profile** button can be used to get an idea of the intensity variation along a line transect.

To use, click the **Image Profile** button and a + will appear.

Move it to the location on the slice you want to examine in more detail and click once.

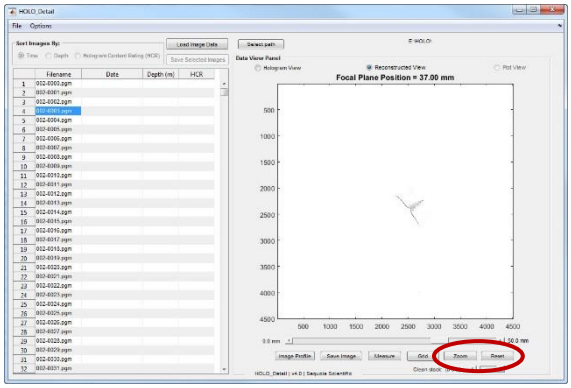
Now drag the cursor to draw a line. Click once to set a point, double-click to mark the end of the transect.

In the image to the left, a transect composed of 3 lines at right angles to each other has been selected.



When you double-click to mark the end of the transect a figure similar to the one on the left will pop up, showing the pixel intensities along the selected transect.

Zoom and Reset Buttons



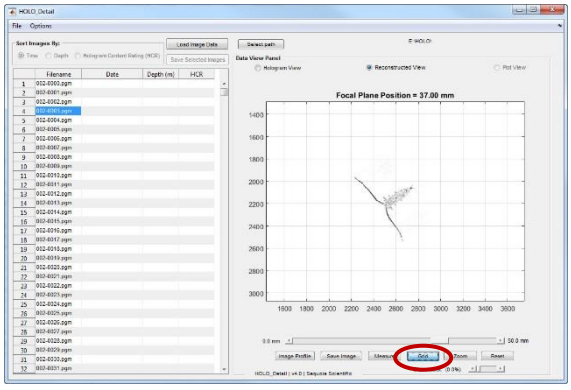
Clicking the zoom button enables you to zoom in on a part of the slice.

Click and drag the cursor to draw a rectangle on the slice and release to zoom to the rectangle.

Note that if you use the arrows to move back and forth between the slices after you have zoomed, the zoom area is retained on the other slices.

Click the Reset button to zoom out again.

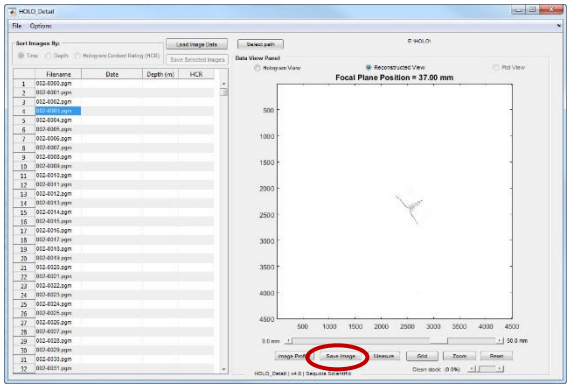
Grid Button



The grid button draws a grid onto the slice. This makes it easier to determine the size of the particles in the slice.

Click the grid button again to turn off the grid.

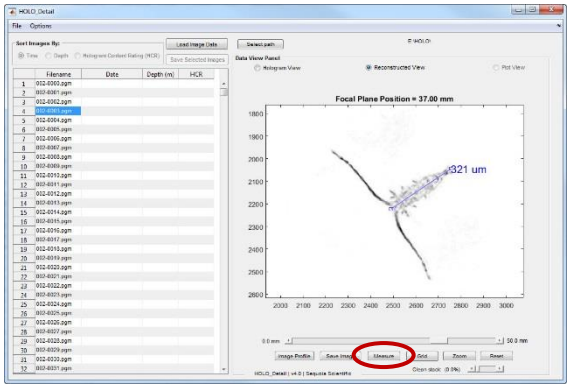
Save Image Button



The Save Image button enables you to save the current view of your slice as an image on your computer.

The image is saved with a .PNG (Portable Network Graphics) extension.

Measure Button

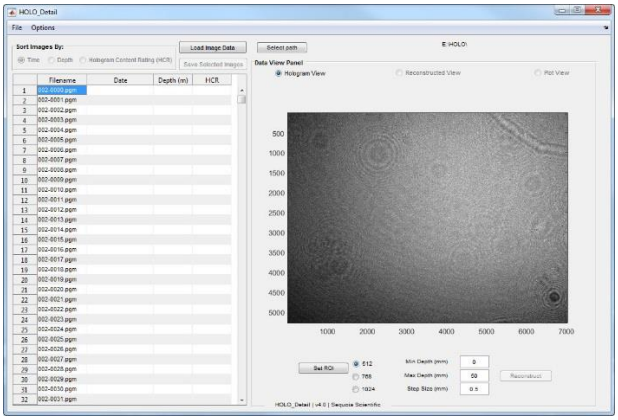


The measure button will allow you to measure objects in the reconstructed slices. After selected the measure button, a + will appear when you mouse over the image.

Click and hold to drag a line across an object in the image. When you release the mouse button, the length of the line will be displayed in microns.

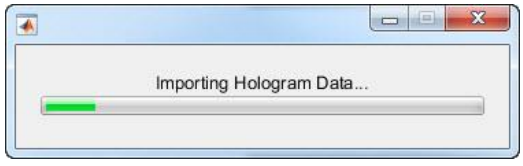
4.6 Holo Detail – Sorting Holograms Based on Depth or Content

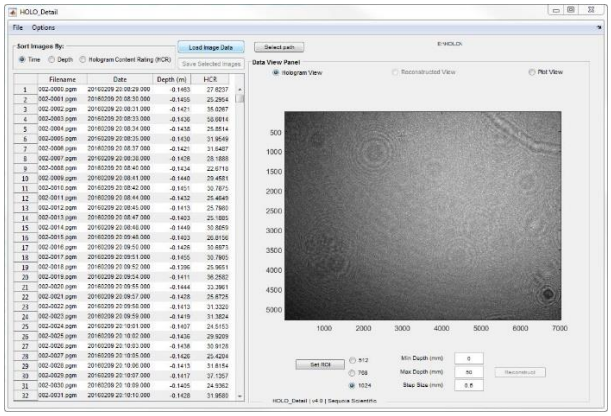
Holo Detail also contains features for sorting holograms based on time stamp, depth, or content. After a long deployment that has generated thousands of holograms, this can be useful tool to sort or organize holograms before processing them with Holo Batch. Sequoia Scientific has develped a method to quickly estimate the particle density in a hologram. We call this metric, the Hologram Content Rating (HCR). It is a not a quantative measure of particle number and is not necessarily correlated with particle concentration. However, it can be used to sort holograms quickly to find the most interesting, or most likely to contain particles.



To enable sorting of holograms, press the 'Load Image Data' button. This will load the hologram meta data and calculate the hologram content rating for each hologram.

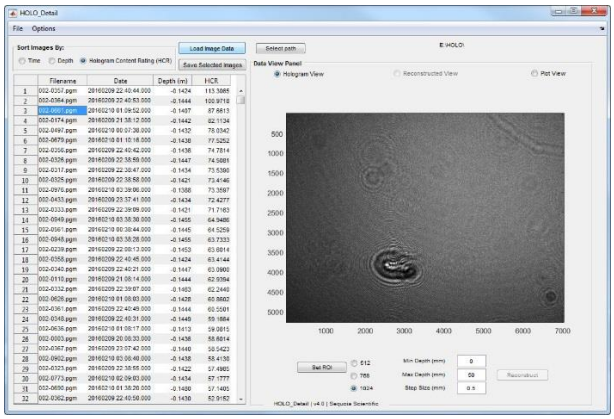
This may take some time, depending on the number of holograms.





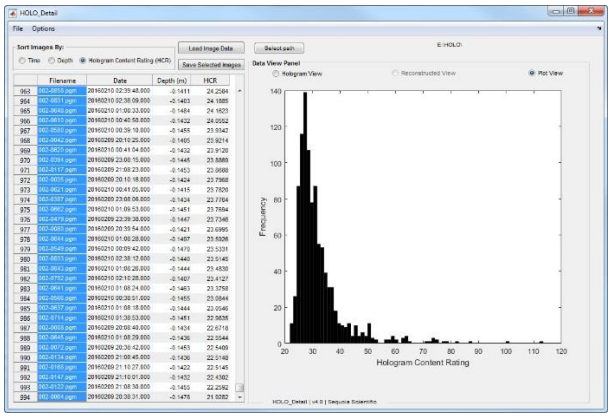
Once the hologram data has been imported, the table will be filled out with the hologram timestamp, depth, and hologram content rating.

The holograms can be sorted based on time, depth, or hologram content rating by selecting one of the ratio buttons under ‘Sort Images By:’



For example, if you wanted to view the most particle rich holograms, you would sort them by Hologram Content Rating. Now the holograms with the highest content rating are brought to the top.

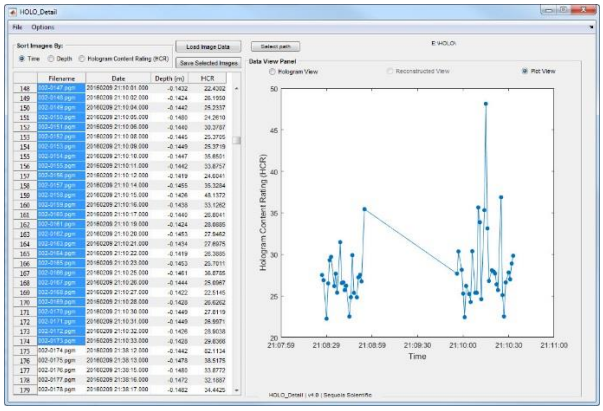
You can now view and reconstruct these holograms as described in the previous section.



Holo Detail also allows you view plots of the hologram meta data.

Switching to ‘Plot View’ under the Data View Panel allows you to select a group of holograms and view plots of hologram content rating as a function of depth, time or frequency.

Changing how the holograms are sorted will change the type of plot generated.



Lastly, you can move a selection of holograms to a new folder by pressing the ‘Save Selected Holograms’ button. This will copy the selected holograms from the current directory, to a new specified folder.

This can usual to further subdivide a group of holograms based on timestamp, depth, or content ration.

5 LISST-Holo2 Field Deployment

5.1 Introduction

The LISST-Holo2 has many features that make it easy to deploy in the field. Start and Stop conditions can be programmed into the instrument in advance. Once the instrument is turned on it will wait for the Start Condition to occur before capturing holograms. It will continue to capture holograms at the programmed rate until the stop condition is reached.

The holograms are stored on solid state drive inside the LISST-Holo2, for later transfer over Ethernet.

Up to four different sampling programs can be pre-programmed into the LISST-Holo2.

The LISST-Holo2 contains a rechargeable 200 watt-hour battery pack, capable of powering the LISST-Holo2 for up to 20 hours of continuous high speed sampling, or longer durations using burst mode or slower sampling speeds.

The sections below describe in detail how to configure, start, and stop the data collection as well as how to offload holograms from the internal drive.

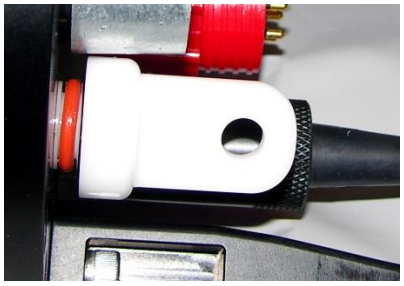
5.2 Charging Internal Batteries

The LISST-Holo2 contains a rechargeable NiMH battery pack. We highly recommend fully charging the batteries before deployment and before long term storage.

Battery charger

The LISST-Holo2 is supplied with a battery charger that connects to the 6-pin connector on the LISST-Holo2 endcap. **Use only the battery charger supplied by Sequoia Scientific.**

The endcap of the LISST-Holo2 has a vent that must be open for the batteries to charge. The vent is the white plastic tab on the LISST-Holo2 endcap. Unscrew the vent until the red O-ring is clearly visible, as shown on the left. Do not open the vent past the point where it turns freely. Opening it too far can damage it.



Open vent

Opening the vent prevents the possibility of pressure build-up inside the housing, in case a charging fault causes the batteries to emit gas. To ensure this safety feature is effective, the LISST-Holo2 charges only when the vent is open. Opening the vent also prevents the LISST-Holo2 from using power from the batteries. To operate the LISST-Holo2 while charging the battery, plug in the external power cable to the 5-pin connector and connect the LISST-Holo2 to its power supply.

IMPORTANT: SUBMERGING THE INSTRUMENT WITH THE VENT OPEN WILL CAUSE IT TO FLOOD AND BE DESTROYED.

- **Only open the vent when charging, and in a dry environment.**
- **Close the vent immediately after battery charging is completed.**

Red clip—a reminder to close the vent

To charge the battery, plug the 6-pin connector from the battery charger into the 6-pin connector on the LISST-Holo2 endcap. **The battery charger has a red spring clip attached to it. Clip this to the vent cover, as shown at left, as a reminder to close the vent when disconnecting the charger.**

The Battery Charger has a red-green LED that indicates the charging status.

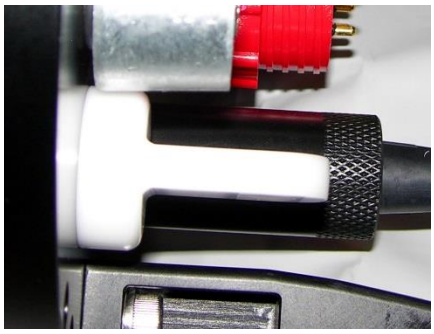
LED Color and Status	Charging Mode
Red and green mixed (orange)	Battery Not Connected
Red and green mixed (orange)	Battery initialization & analysis
Red	Fast Charge
Green with intermittent red flash	Top-off Charge
Green	Trickle Charge
Alternating red and green	Error

After connecting the Battery Charger to the LISST-Holo2 and connecting to AC power the charger will initialize and evaluate the battery before charging will being. This takes about 5 to 10 seconds. During this time, the LED will be a mix of red and green before turning to either solid red or green.

Observe the charger LED to confirm that it turns red, indicating the start of normal fast charging.

- **If fast charging does not start, check that the vent is open.**
- **If the vent is open but fast charging still doesn't start, also connect the external power supply through the 5-pin bulkhead connector** (this is necessary when the batteries are so deeply discharged that the battery supervisor electronics have turned off)

Charge times vary depending upon the state of charge in the battery. Fully depleted batteries will take about 6 hours to charge. Do not leave the charger connected longer than 12 hours.

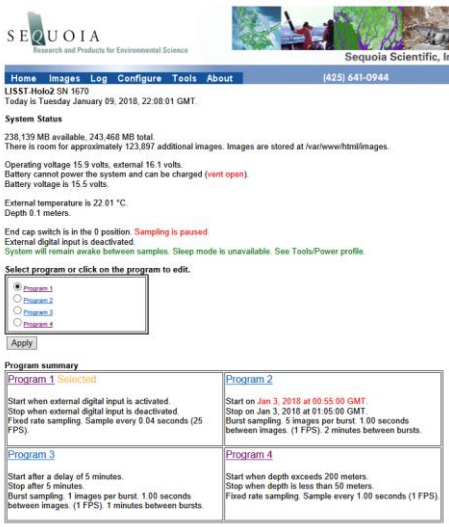


Fully-closed vent

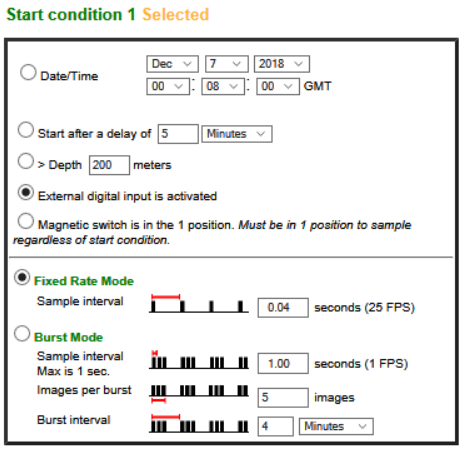
When the charge is complete, remove the charger and close the vent completely, as shown at left. The vent should be screwed down till it is finger tight. **Use only hand strength—no tools—to tighten the v**ent Over-tightening can break the plastic part and cause leakage into the housing.

5.3 Programming

To configure the Start and Stop conditions you must connect to the instrument using the Ethernet connection as described in Sections 3.2 and 3.3.



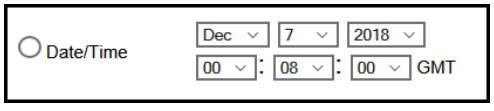
Clicking on the Home tab will show a summary of the four sets of sampling conditions. Each of the four sets can be configured differently. To set up a sampling program click on the 'Program #' hyperlink.



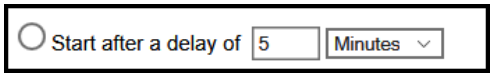
The Start Condition, Sampling mode and rate, and the Stop Condition will need to be set.

To set the Start condition choose between a Date/Time start, Delayed Start, Depth Start, External Signal 1, External Signal 2, or the Magnetic Switch.

To set the sample mode, choose between Fixed rate and burst sampling.

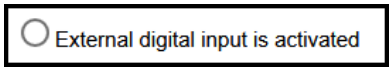


Date/Time start will wait until the selected GMT time is reached before sampling is started and the magnetic switch is in the 1 position. If it is past the specified time the sampling will start immediately if the magnetic switch is in the 1 position. Be sure to check the homepage to make sure your LISST-Holo2 has the correct GMT time.

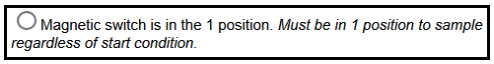


The Delay Start will start after the specified delay. The delay must be an integer number and can be specified as Minutes or Hours. In this example, the LISST-Holo2 will start sampling 5 minutes after the magnetic switch has been turned to '1' (On) position.

The Depth Start will wait until the depth is greater than the specified value before starting. In this example, the LISST-Holo2 will start sampling once the depth exceeds 200 m and the magnetic switch is in the 1 position.

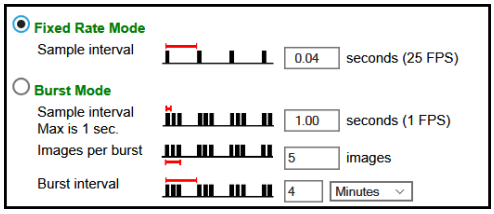


The External Digital Input will wait for the voltage on the selected External input located on the Auxiliary 6-pin connector to greater than 5V. Maximum recommend input voltage is 12V.



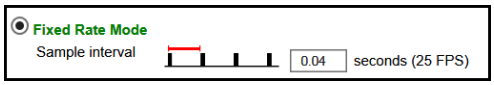
The Magnetic switch option will start sampling when the Switch Lever on the endcap is in turned to the 1 position.

NOTE: ALL PROGRAMS MUST BE STARTED BY TURNING THE MAGNETIC SWITCH TO THE 1 POSITION.

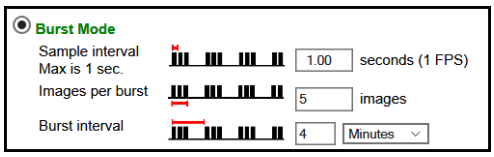


The sampling rates and modes also need to be selected. There are two modes:

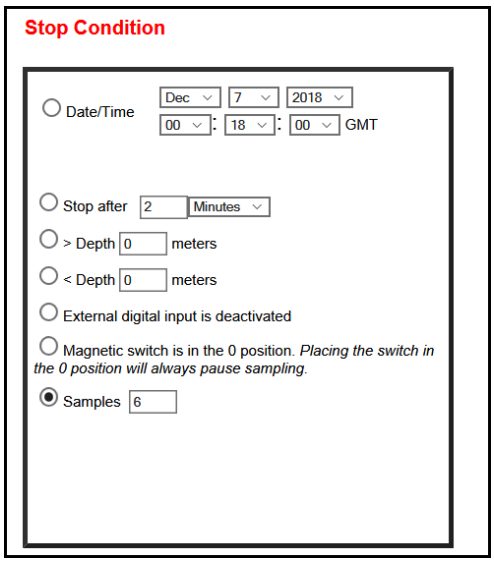
- Fixed Rate Mode and
- Burst Mode.



The Fixed rate mode will take one hologram at the specified interval. Holograms can be taken at a frequency of up to 25 Hz (0.04 second interval).



The Burst Mode can be used to take a burst of holograms at a fixed time interval between the start of each burst. The maximum sample rate is 25 Hz (0.04 second sample interval).



The Stop Conditions also have multiple options: Date/Time, Fixed Duration, Greater than or less than Depth, External Digital Input, Magnetic switch, or fixed number of Samples.

The Date/Time Stop is used to stop collection at a particular time and date. The date and time must be in GMT.

The Stop after setting will stop the sampling after the specified amount of time from the start of data collection.

The > Depth value will stop when the depth is deeper than the specified depth.

The < Depth value will stop when the depth is shallower than the specified depth.

The External Digital Input setting will stop collection when the external input voltage signal is less than 0.8 volts.

The Magnetic Switch option will cause sampling to stop when the Switch Lever on the endcap is in the 0 position

The 'Samples' setting will stop data collection when the specified number of samples has been collected.

After making changes to the settings press on the Apply button to save the settings and refresh the page.

All four sets of start and stop conditions can be individually programmed.

Selecting the start/stop program using the PC

When the four Start and Stop conditions have been programmed, you must select which program you want to use by selecting the appropriate radio button and clicking apply.

5.4 Wake up / power up LISST-Holo2

If the system is in sleep mode (no endcap LED flashes), move the Switch Lever to the 1 position and then quickly back to the 0 position. This will wake up the instrument.

The LISST-Holo2 will boot up and be ready in 40 to 60 seconds.

When the green LED is flashing every few seconds the instrument is awake and ready to start sampling.

5.5 Start Data Collection

To start the data collection program, move the Switch Lever to the 1 position. The LED on the endcap will change from green to red indicating that sampling has begun.
It will blink slowly until the start condition is reached and it will be on continuously during the acquisition of a hologram.
It will return to green when the stop condition has been reached.

5.6 Stop Data Collection

Data collection will continue until the selected Stop Condition is reached. To Stop the data collection at any time, move the switch to the 0 position. Within a few seconds the LED on the endcap should change from Red to Green.

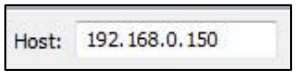
If the data collection has already stopped the LED will be blinking green regardless of the switch position. However, the Switch Lever should always be placed in the 0 position when sampling is complete.

5.7 Downloading Raw Holograms

The raw holograms are saved on the internal solid state drive of the LISST-Holo2.

Downloading and deleting from LISST-Holo2 internal drive

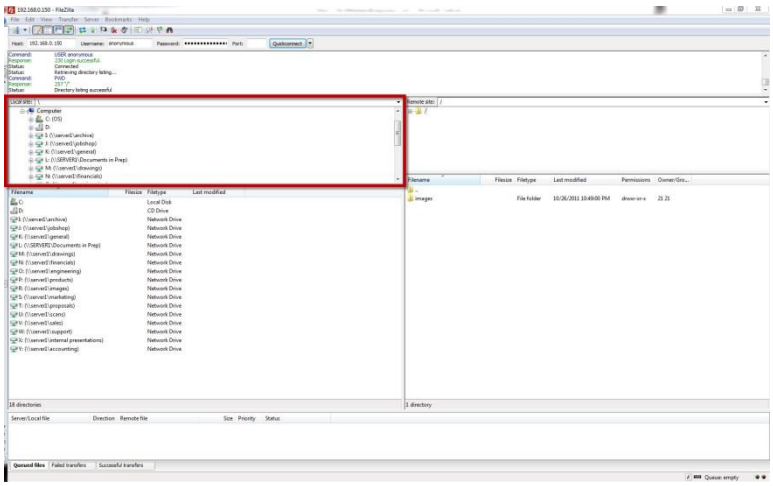
To download holograms from the internal drive you will need an FTP utility like Filezilla (http://filezilla-project.org). When you have started the FTP application, you will need to specify a host. To ensure the LISST-Holo2 remains awake while downloading images, you should disable automatic sleep on the Tools page of the Holo2 webpage. If sleep is not disabled, the Holo2 will enter sleep mode after 10 minutes.



The host is the IP address of the LISST-Holo2, typically **192.168.0.150** unless you have changed it to another IP address (see section 8.5).

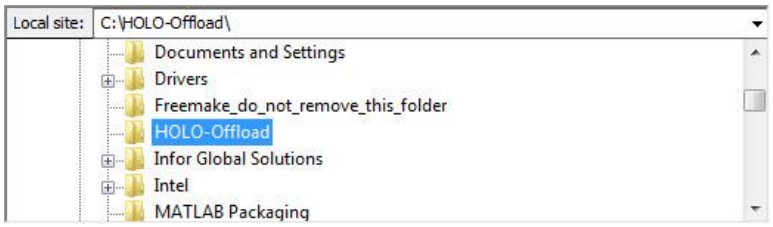
Leave the username and password blank

Click the Quickconnect button to start the FTP transfer.

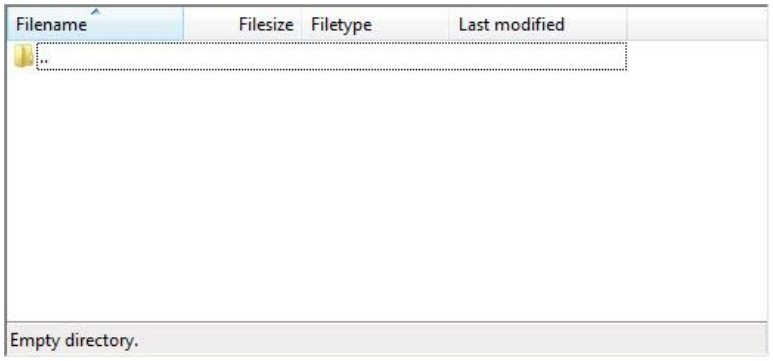


Filezilla will connect to the LISST-Holo2 and display its contents in the bottom right window. The hologram files are in the directory /var/www/html/images.

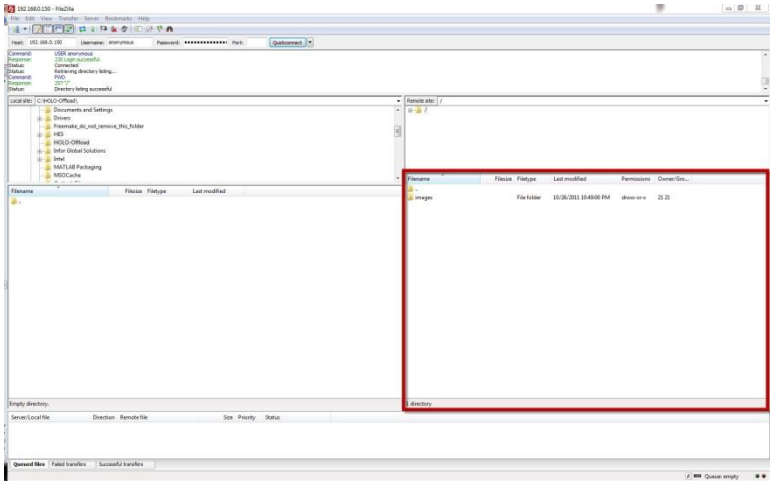
To the left is the destination folder selection. Navigate to the desired destination folder.



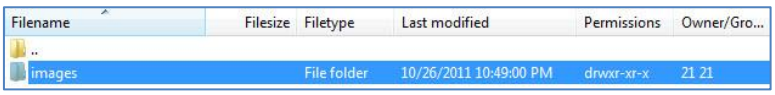
In this example, we will offload to a folder on the C drive called HOLO-Offload.



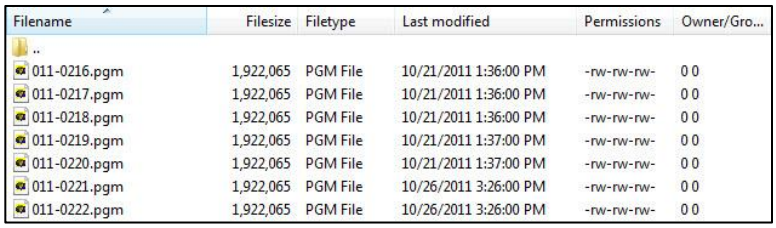
Note that the destination directory window is empty



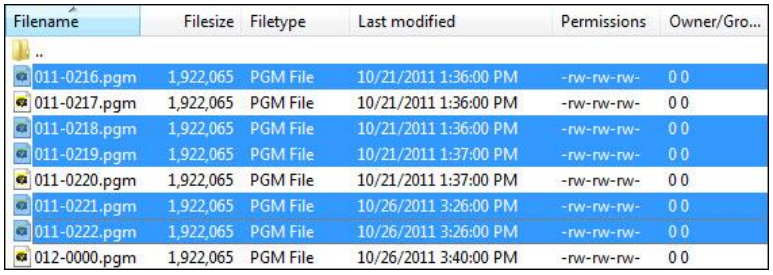
Focus on the directory listing in the bottom right window.



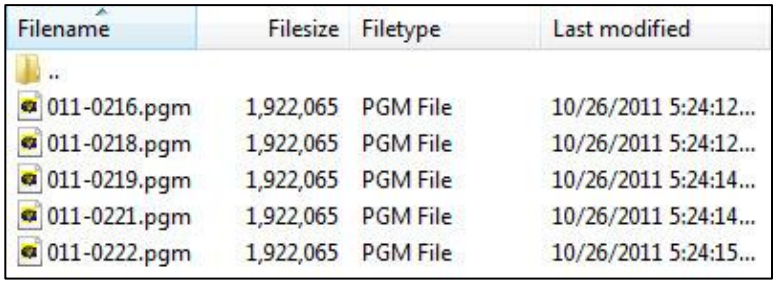
Double-click the folder called **images**.



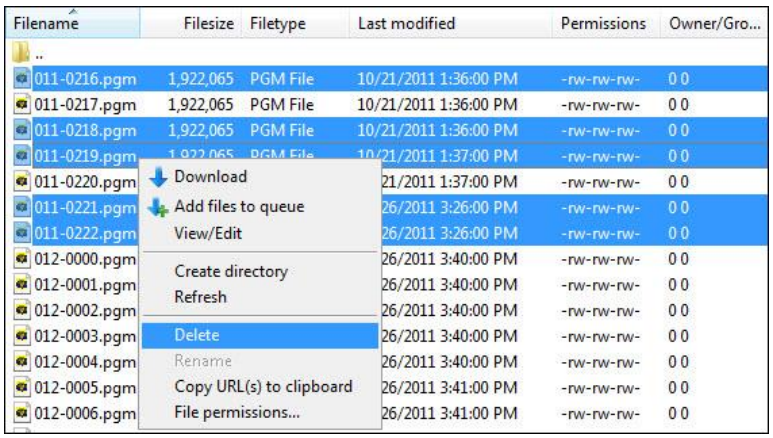
The holograms on the internal drive are now displayed.



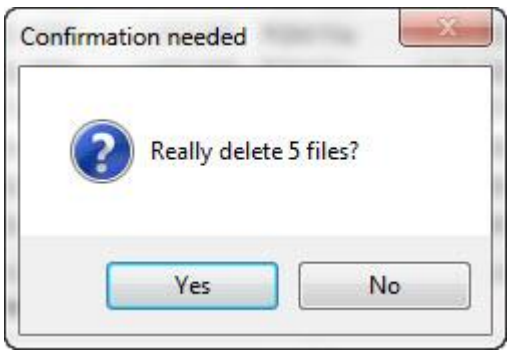
Select the holograms you wish to offload or delete. Use the SHIFT key to select a range of holograms. Use the CTRL key to select several individual holograms. Here 5 holograms have been selected.



Now drag the selected holograms straight across to the destination folder window on the left side of the screen. The selected holograms will then transfer to the destination folder



To DELETE the holograms, right click on the selected holograms on the **images** drive on the LISST-Holo2 and select Delete.



You will be asked to confirm the deletion.

The holograms are then deleted from the internal drive on the LISST-Holo2.

WHEN THE HOLOGRAMS HAVE BEEN DELETED THEY CANNOT BE RECOVERED!

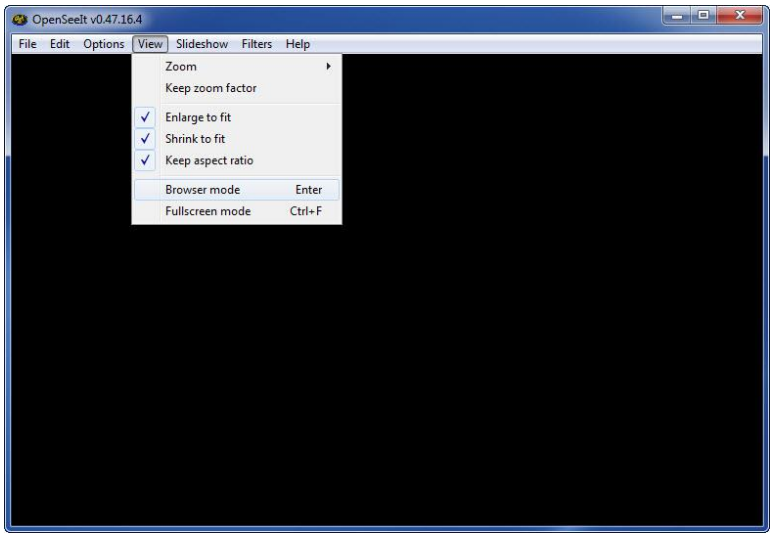
5.8 Viewing raw holograms in OpenSeeIt

To quickly view the raw holograms, use the Browser mode of the OpenSeeIt software.

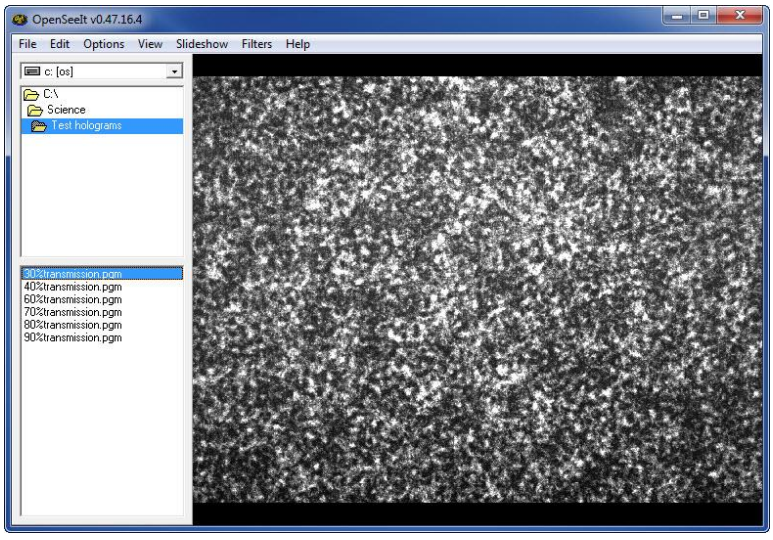
If you have set OpenSeeIt to be used for all PGM files you can simply double click on one of the holograms in order to see it in OpenSeeit.

If you press <Enter> within OpenSeeIt when a hologram is displaying the View mode changes to Browser mode.

Otherwise follow the instructions below.

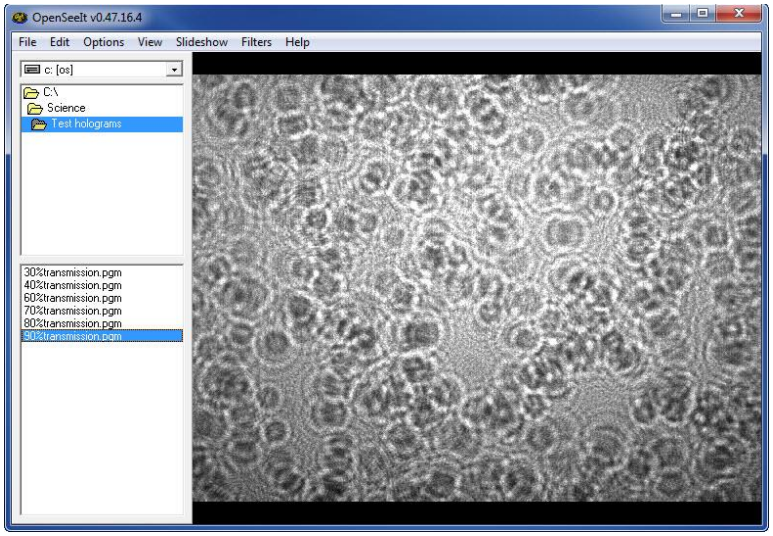


Open up OpenSeeIt and select Browser Mode from the View Menu.



Navigate to the folder where your holograms are stored. They will all be displayed in the bottom left of the OpenSeeIt window. Use up/down arrow keys to scroll through the holograms. You can use this functionality to quickly determine which holograms will be suitable for processing and which will not.

The hologram on the left will not process well, because there are no visible interference patterns (concentric rings). This is due to the particle concentration being too high.



This hologram will process well, because the interference patterns are clearly visible and distinct.

5.9 Next Deployment or Powering Down

There is no need to connect the Ethernet cable and establish communication with the instrument unless the settings for the Start and Stop conditions need to be changed or holograms need to be downloaded. Moving the Switch Lever to the '1' position will start the current sampling program.

For short term or long term storage of the LISST-Holo2 it should be put into Sleep mode. The instrument will automatically go into lower power sleep mode after 10 minutes of inactivity if the automatic sleep has not been disabled on the Tools page. You can also manually put the LISST-Holo2 to sleep using the Sleep button on the Tools page. The switch lever should be left in the '0' position and it is highly recommended that the internal batteries be fully charged before storing the instrument. The Battery Vent should be closed during storage or anytime the batteries are not being charged.

6 Reviewing Instrument Info and Other Settings

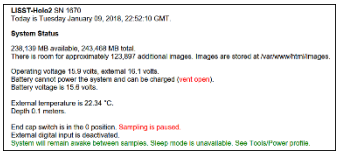
6.1 Intro to Other Settings

In addition to the Start and Stop Conditions there are other parameters that can be changed using the Ethernet connection. This section will discuss some of the additional parameters that can be accessed from the home page.

6.2 Home page

The Home page shows the System Status, Selected Program and Programming Information. It also shows Start and Stop conditions for the four different sampling programs.

System Status



The System Status shows the available memory on the internal flash drive, input voltage, current temperature and depth, endcap switch position, and the state of the two digital inputs.

Select program

The Select Program buttons lets the user select which program should control the LISST-Holo2 sampling. Up to 4 programs can be loaded in the LISST-Holo2.

In the example to the left, Program 1 is selected. If another program is to be selected, click the appropriate radio button and then click the Apply button.

Programming Information

For details on the start/stop conditions for the four different programs, see section 5.3.

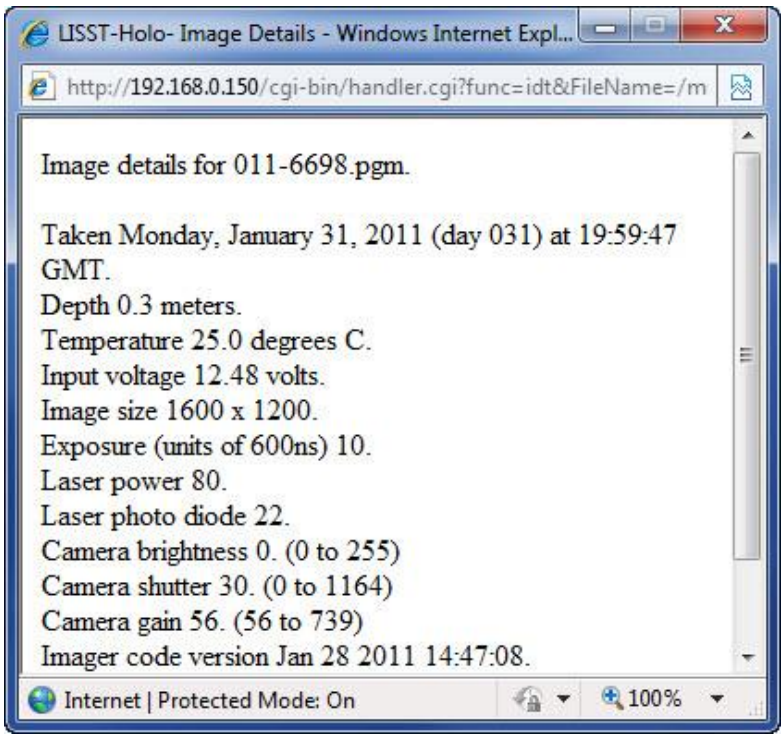
6.3 Images page

The Images page shows a listing of the holograms saved on the internal solid-state drive.

The hologram file name is a hyperlink to the file on the internal drive of the LISST-Holo2. Click on the file name to download the file to a temporary folder and open the file in OpenSeeIt.

To copy the file to a specific folder, right click on the file name and choose “Save Target as” or “Save Link as” and then enter a folder to store the file to.

To see specific information about the hologram such as date and time, depth, and temperature click on the Details button.



The Details Button opens up a 2nd window showing the details.

Click the Delete button to delete a hologram. You can only delete one hologram at the time using the Delete button.

To download or delete multiple holograms from the internal drive you will need to use an FTP utility like Filezilla (http://filezilla-project.org) as described in section 5.7.

6.4 Log page

The LISST-Holo2 writes information to a file as it is operating. This file contains information that can be helpful in troubleshooting problems and it can be sent to Sequoia for evaluation. The Log option of the main menu allows the user view the contents of the log file.

6.5 Configure page

Selecting Configure on the home page main menu will bring up a window listing many parameters and allows their values to be changed.

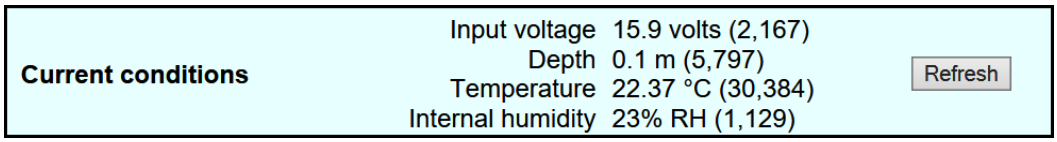
Values on this page should not be changed without detailed knowledge of the parameter's use or without detailed instructions.

By default, this page has restricted access and the user should NOT change any of these values without being asked to do so by Sequoia.

6.6 Tools page

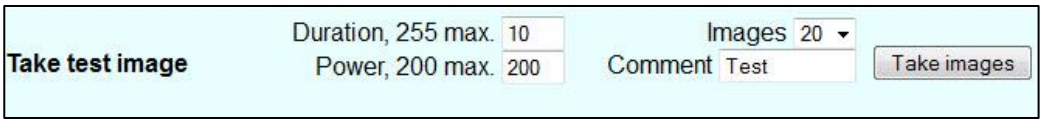
Selecting Tools on the main menu brings up a window that allows the user to take a test image and to change additional parameters such as IP address, send Log file for analysis, check for firmware updates, synchronize time with standard time, and other settings. These options are described in detail in the following.

Current conditions



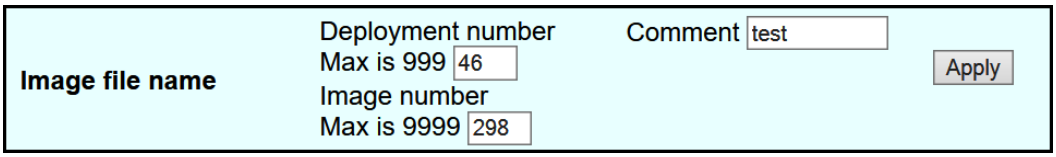
The current voltage, temperature, depth and internal humidity are displayed. Also displayed are the digital counts. Click the Refresh button to update.

Take test image



To take a manual hologram, click the Take images button. You can take up to 20 holograms by selecting the number using the drop-down menu. The holograms are collected at about 10 frames per second. If you enter a comment, it will display when reviewing the image details (see section 6.3). Do not change the duration or power settings.

Image file name

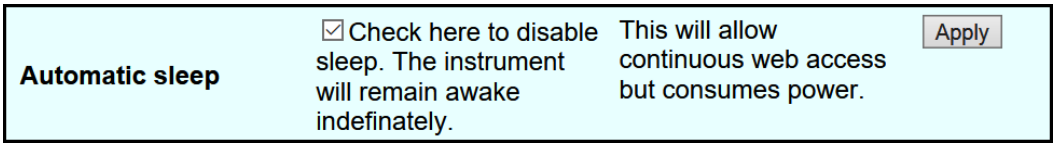


You can force the LISST-Holo2 to start at a certain deployment number or image (hologram) number. You can also enter a comment that can be used later to identify the holograms, e.g. the location. Remember to click the Apply button.

Sleep or reboot

Click the Sleep button to put the LISST-Holo2 to sleep. When the LISST-Holo2 goes to sleep you will lose connection with the instrument.
Click the Reboot button to reboot the LISST-Holo2. If you reboot you must re-enter the LISST-Holo2 IP address in your browser window to re-establish communication.

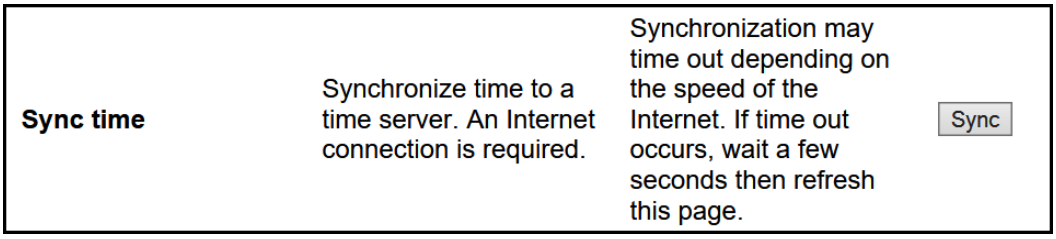
Automatic Sleep



If the Automatic Sleep Disable check box is checked as in the example above, the instrument will not go to sleep between samples. Use this setting if the instrument is connected to external power. You should UNCHECK this checkbox if the LISST-Holo2 is only powered by its internal battery. This preserves battery life.

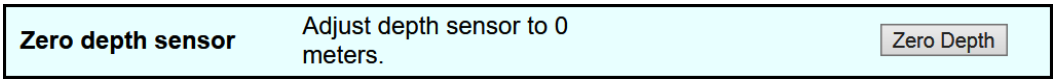
The Sleep button on the Home page will disappear if the checkbox is checked.

Sync Time



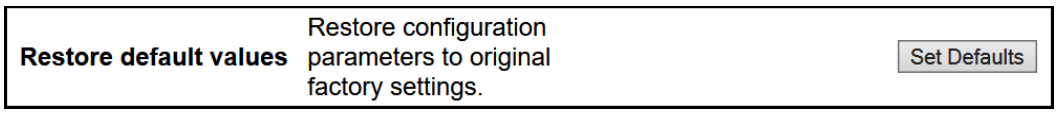
Click the Sync button to synchronize the LISST-Holo2 to a time server.
The LISST-Holo2 must be connected to the Internet to synchronize the time.

Zero Depth Sensor



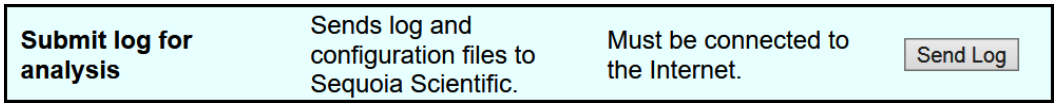
Click the Zero Depth button to automatically adjust the offset on the depth sensor.

Restore default values



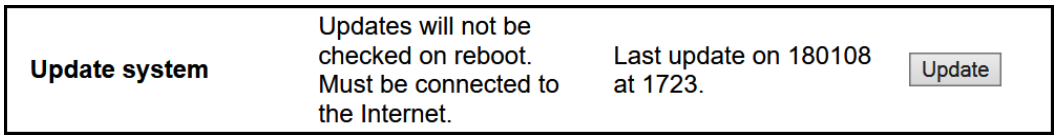
Clicking the Set Defaults button will restore ALL settings to factory defaults including sampling program settings.

Submit log for analysis



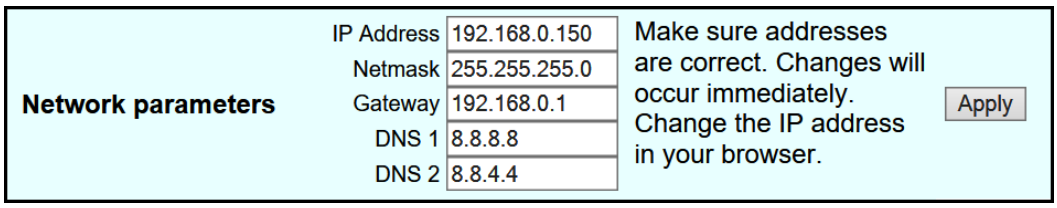
Clicking the Send Log button will send the deployment log to Sequoia for troubleshooting. The LISST-Holo2 must be connected to the internet and it must be rebooted after the Send Log button has been clicked for the log to transfer.

Update System



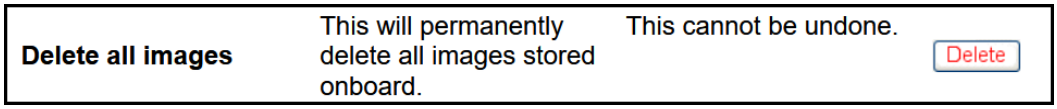
If you click the Update button, the LISST-Holo2 will check for software updates the next time it reboots. This requires connection to the Internet. Use the Reboot button, above, when ready.

Network parameters



Use Network parameters to change the IP address of the LISST-Holo2. Click Apply when you have changed it, but make sure that it is correct. The changes will take effect immediately, so you will need to enter the new IP address in your browser to continue working with the LISST-Holo2.

Delete all images

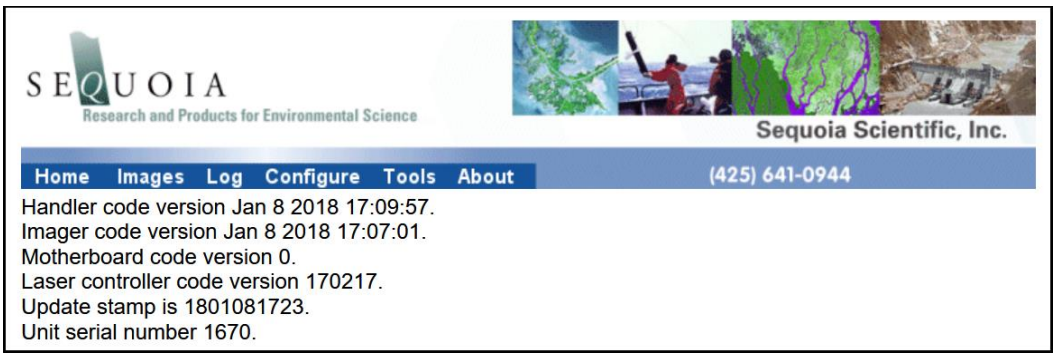


Click the Delete button to delete **all** files on the internal hard drive of the LISST-Holo2. You will be asked to confirm. If you confirm, deletion will begin immediately, and takes only a few seconds.

Parameters below this point should be adjusted only after consultation with Sequoia Scientific, Inc.

Do not use controls below the yellow warning unless instructed by Sequoia Scientific.

6.7 About page



The top of the About page contains information about the hardware and firmware loaded in the instrument, as well as the serial number of the instrument.

Below this section are a few FAQ's, as well as links to the free OpenSeeIt and Filezilla software.

7 Handling, Cleaning, Maintenance and Storage

7.1 Handling

When handling the instrument, please remember that the LISST-Holo2 is a high-precision optical instrument.

Avoid mechanical shocks and impact to the housing, as this may cause misalignment of the optical parts.

The instrument pressure housing should not be opened.

7.2 Cleaning

The glass windows of the instrument can be scratched.

Do not use abrasive cleaners on glass or anodized surfaces.

When cleaning windows be careful not to drag and hard particles such as sand across the windows.

Use glass cleaner or liquid dish soap to clean windows.

7.3 Maintenance

Make sure that the windows are as clean as possible. The windows can be cleaned with Windex, but often a mild, lukewarm, soap solution is the best for cleaning optical windows. Dip a finger in the soapy solution and gently rub the windows. Rinse several times with clean, particle-free water.

Lubricate the connectors regularly with silicone spray or a light coating of silicone grease. Avoid contaminating the windows with lubricant. **Do not use any petroleum-based lubricant.**

7.4 Storage

Clean the LISST-Holo2 with fresh water and dry it before storage.

Store in a clean dry place or inside the provided ship case.

Make sure the caps for the connectors are in place before storage.

The instrument should be disconnected from power when not in use. The internal batteries should be fully charged and the switch lever should be in the ‘0’ position.

8 Ethernet Communications

There are four method for connecting to the LISST-Holo2:

1. Wi-Fi connection to the supplied router
2. Wired connection to the supplied router
3. Direct wired connection to a computer
4. Wired connection to a workplace network

When using any of these methods, check that the LISST-Holo2's end cap LED is flashing green every 5 seconds, to indicate that it is ready for communication.

8.1 Wi-Fi connection via supplied router

The LISST-Holo2 is supplied with a wireless router, set up by Sequoia to enable the following procedure.

1. Connect power to the router, using its AC adapter.
2. Using the blue ethernet cable adapter from the LISST-Holo2, connect to one of the ethernet ports on the router (NOT the yellow "Internet" port).
3. Use the standard process for connecting your computer to a Wi-Fi network. The router will appear as a wireless network with the name **LISST-Holo2**. The network password is **manyholograms**.
4. You should now be able to connect to the instrument through a browser, at http://192.168.0.150 (unless its address has been changed).

8.2 Wired connection via supplied router

If your computer has an Ethernet connector available, you can use an Ethernet cable to connect directly from the computer to the router.

1. Connect power to the router, using its AC adapter.
2. Using the blue Ethernet cable adapter from the LISST-Holo2, connect to one of the ethernet ports on the router (NOT the yellow "Internet" port).
3. Connect your computer's Ethernet port to another port on the router (again, not the yellow port).
4. Your Windows computer should automatically connect to the router and allow you to reach the LISST-Holo2 at http://192.168.0.150.
5. If your computer does not automatically detect the connection, check that its IP Settings include Automatic IP assignment (DHCP).

8.3 Direct wired connection from computer to LISST-Holo2

This method requires manually changing the network settings on your computer. If your computer is normally connected to a wired network, this will likely block access to that network.

1. Connect the blue ethernet cable from the LISST-Holo2 to the ethernet port on your computer.
2. From the Windows menu, open the Control Panel.
3. Under **Network and Internet**, click on **View network status and tasks**
4. You should see: Connections: Ethernet . Click on **Ethernet** to see the Ethernet Status window.
5. Click the Properties button, to see the **Ethernet Properties** dialog.
6. In the list of connections double-click on **Internet Protocol Version 4 (TCP/IPv4).**
7. In the Internet Protocol Version 4 (TCP/IPv4) Properties dialog, note the current settings. Save them so you can restore them when you are finished communicating with the LISST-Holo2. Then enter the following:
   IP address: 192.168.0.1
   Subnet mask: 255.255.255.0
   Default gateway: 192.168.0.0
   Preferred DNS server: 192.168.0.1
   Alternate DNS server: 192.168.0.2
8. Click OK and close the dialogs.

8.4 Wired connection to a workplace network

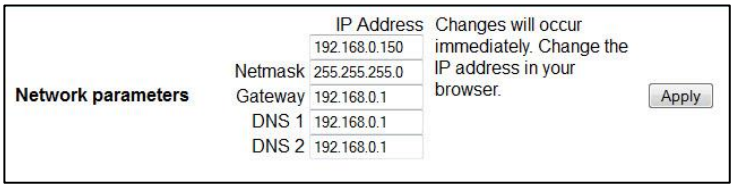
If the LISST-HOLO2's default address of 192.168.0.150 is compatible with your local network, you can simply connect it through Ethernet. If not, you may need to change its IP address and other IP parameters as described in the following section. Consult with your local network administrator for details.

8.5 Changing the LISST-Holo2 IP settings

WARNING: use great caution if modifying the instrument's IP settings. In case of error, it may be very difficult to restore communication!

To change the IP address of the LISST-Holo2 use the web interface. Connect as described in section 3.2 and go to the Tools page of the LISST-Holo2 web interface.

Scroll down until you get to Network Parameters.



Change the IP address to the desired address and click the Apply button. The changes will take effect immediately, so you will need to enter the new IP address in your browser to continue working with the LISST-Holo2

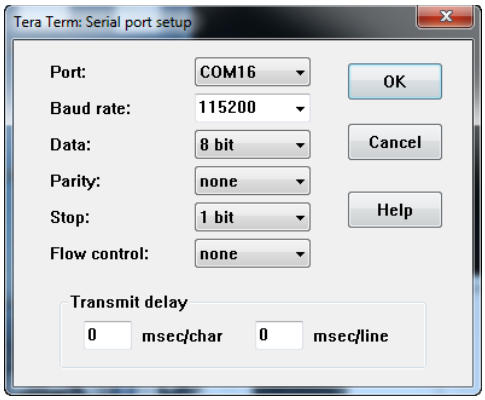
8.6 Recovering Lost IP Address Using the Serial Port

In order to communicate with the LISST-Holo2 through the web interface, you must know the IP address of the instrument.

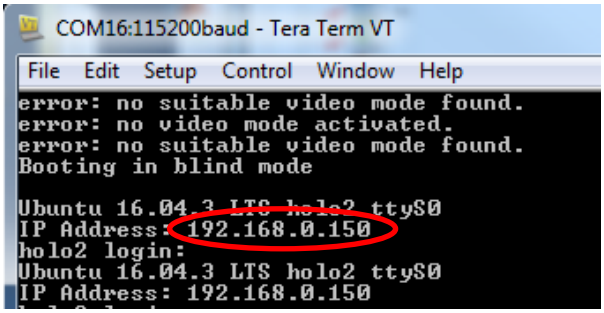
If you do not remember the IP address if your instrument, you can look up the IP address using the instrument serial port.

Connect the power cable to the 5-pin connector on the LISST-Holo2. Plug the USB end of the cable into your computer. You may need to wait a few minutes for your computer to install the USB to serial adapter driver.

If the LISST-Holo2 is not already awake, toggle the magnetic switch to the one position, then back to zero position to wake the instrument.



Connect to the LISST-Holo2 using a terminal program such as HyperTermal, TeraTerm, or PuTTY. Connect to the instrument using the settings shown on the left, however, change the 'Port' field to the appropriate port on your computer.



Once connected and the LISST-Holo2 is fully booted, press the enter key several times.

You should see a response similar to the one on the left. The 'error' messages displayed in the terminal are normal and should be ignored. The IP address will be listed before the login prompt.

9 FAQs

What is a hologram?	Fundamentally, holograms are 3-D images that have first been projected, and then stored on a 2-D surface. In the type of holograms captured by the LISST-Holo2 (so-called in-line holograms), a laser beam is emitted and a small part of it is being scattered by the particles in the water. The part of the laser beam that is not being scattered by particles interferes with the scattered light. This creates an interference pattern - a hologram. The interference pattern is captured by a small digital camera. Upon processing of the captured interference pattern (i.e. the hologram), it is possible to recover the original image that created the hologram. Thus, the LISST-Holo2 does NOT capture and store an image of the suspended particles. It is the hologram of the particles that is stored. During data processing, the image of the suspended particles is reconstructed from the hologram. Subsequently standard image processing methods can then be applied to get information about particle shape, size, area, etc.
I connected to the LISST-Holo2 with my web browser, but now it says "500 – Internal Server Error", or it doesn't respond at all.	Most likely this is because the LISST-Holo2 is asleep, or is busy collecting holograms. If the indicator light on the end cap is rapidly flashing, it is sampling; set the switch lever to 0 to stop it. If the light is dark, it is asleep; try toggling the switch lever from 0 to 1 and back. If the LISST-Holo2's indicator is flashing green every 5 seconds, it should be ready to communicate. Check the connections to your network.
How do I wake the LISST-Holo2 when it is asleep?	Toggle the white switch lever from 0 to 1 and back. That will wake it without starting logging. If you leave the switch in the 1 position, the logging program will start.
Toggling the switch didn't work. Why not? What now?	When the internal batteries are very low on charge, too low even to boot up, the LISST-Holo2 will go into deep sleep. To wake from deep sleep, you must apply external power, either from the battery charger (through the 6-pin connector) or the separate power supply (through the 5-pin).
The LISST-Holo2 went to sleep while I was offloading files. How do I fix that?	Go to the Tools page and look for Automatic Sleep. Check the box that disables automatic sleep, and click the Apply button.
Why can't the LISST-Holo2 be used when the flow speed is more than 2 m/s?	It takes 2 microseconds to capture the hologram using the digital camera. The image captured by any digital camera is made up by a large number of pixels. If the particles in the water move more than one pixel during the time it takes to capture and store the hologram, the quality of the reconstruction images will be affected. For the pixel size used in the LISST-Holo2, a flow speed of more than 2 m/s will begin to blur the hologram. Consequently, if the LISST-Holo2 is used on a profiling platform, e.g. a CTD, the profiling speed must be 2 m/s or less. Likewise, if the LISST-Holo2 is deployed on a mooring or tripod, the current velocity around the instrument must be less than 2 m/s.
Why should I connect the LISST-Holo2 to the Internet?	Connecting the LISST-Holo2 to a local area network that is connected to the Internet has benefits. The first benefit is that the instrument is accessible from different locations across the network. Connecting the instrument to the Internet has other advantages such as checking for firmware updates, syncing the time with international standards, and transmitting log files to Sequoia. It is not necessary to have the instrument connected to a network or the Internet but it can be useful when needed.
How do I connect the LISST-Holo2 to the Internet?	The LISST-Holo2 comes configured with a pre-selected IP address, netmask, gateway, DNS 1, and DNS 2. These settings will work with the provided wireless router but they will probably not work with your existing local area network. Because the LISST-Holo2 requires a static IP address, an IP address must be selected so as not to interfere with other computers on your network. It is highly recommended that users work with their network administrators to determine the proper settings for their network. The new value can be entered on the Tools page (see section 8.5). Be sure to double check the values before pressing the Apply button on the Tools page. Once the Apply button is clicked the IP address will change immediately and you will need to log into the new IP address before communication can be re-established. If incorrect values are entered it is possible to change them manually through serial port communication. Contact Sequoia for details of this procedure.
Does the LISST-Holo2 support DHCP?	No. The LISST-Holo2 does not support DHCP (Dynamic Host Configuration Protocol).

What is the maximum sample rate for the LISST-Holo2?

The maximum sample rate for the LISST-Holo2 is 25 Hz (25 holograms per second).

Can I connect the LISST-Holo2 directly to the Ethernet port on my PC?

Yes, unless your PC has very old Ethernet hardware. For instructions, see “Direct wired connection from computer to LISST-Holo2” on page 47.

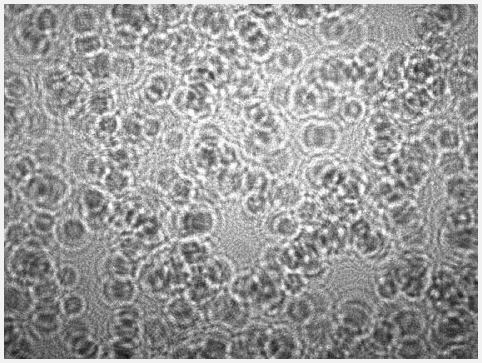
What is the maximum concentration that the LISST-Holo2 can be used with?

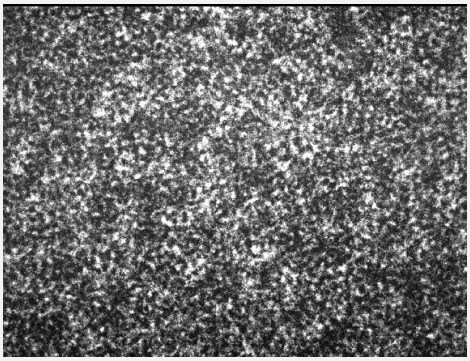
The concentration limits for a LISST-Holo2 are roughly as tabulated in the table below. See also this article on Sequoia’s website: http://www.sequoiasci.com/article/what-are-the-concentration-limits-for-the-LISST-Holo2/ for a more thorough explanation.
The optional Path Reduction Module can be used to shorten the optical path which will allow up to a factor of 5 increase in the maximum concentration.

Mean Particle Diameter		Wentworth Grades	Maximum Concentration
[µm]	[phi]		[mg/l]
1.95	9.00	Clay	8
3.9	8.00	Very Fine Silt	15
7.8	7.00	Fine Silt	31
15.6	6.00	Medium Silt	62
31.25	5.00	Coarse Silt	123
62.5	4.00	Very Fine Sand	247
125	3.00	Fine Sand	494
250	2.00	Medium Sand	987

How do I tell if the suspended sediment concentration is too high?

If you cannot see any interference patterns (concentric rings) in the hologram, the concentration is too high and you most likely won’t get any sensible data out of the hologram when processing it. The image on the left has visible concentric rings and will process well. The image on the right has too many particles and will not produce good images of particles.





Why are my holograms completely black or white?

Dark or saturated holograms may be cause by incorrect laser and camera settings on the instrument. On the ‘Tools’ page of the web interface, select the ‘Set Defaults’ button to restore the instrument settings back to the factory settings. Reboot the instrument and try collecting images again. If the problem persists, contact Sequoia Scientific for further instruction.

10 Endcap LED Status Light Indicators

The LISST-Holo2 connector end cap has a red-green-blue LED that indicates its state and activity.

LED state	What the LISST-Holo2 is doing
Dark	System is asleep
YELLOW (green and red mixed) flashing twice per second	System is booting up. This typically takes 40 to 60 seconds.
Short **GREEN** flash every 5 seconds	Idle; ready for Ethernet communication.
GREEN followed by **BLUE**, every 2 seconds	A sampling program is active, but is waiting for the start condition to be met.
GREEN flashing continuously twice per second	A sampling program is running, and collecting images at the programmed rate (the flashing speed is not related to the rate of images). Ethernet communication is not available.
RED flashing continuously once per second	Error condition; mostly likely, the image memory is full.
The LED may also show brief blue, red or white flashes when changing states, for example when first waking from sleep, or when starting sampling	

11 Technical Specifications

1) LISST-Holo2 with internal NiMH battery
2) Instrument stands
3) Large Clear box with battery charger, power supplies and power/communication cable
4) Small Clear Box with Test chamber, cleaning supplies and Clamps
5) Box with Manual, USB memory card with software, wireless router and Ethernet cables

11.1 General Features

- In-situ digital in-line holography
- Self-contained with internal data storage and rechargeable battery
- Ethernet connection to PC for programmable data collection—web browser interface
- Power via internal battery pack or 12 - 24V (18 – 24V preferred) external power source (cable and 19V power supply provided)
- Programmable data collection
- Optical path length: 50 mm standard
- Sample volume: 1.86 cm3
- Data processing yields in-focus particle images and volume distribution

11.2 Parameters Measured and Derived

- Particle images for observation and classification
- Particle volume distribution
- Temperature
- Depth

11.3 Particle Size and Concentration

- Size range: 25-2500µm equivalent spherical diameter with 4 µm feature resolution
- Concentration range: Beam attenuation coefficient of 0-4 m^{-1}; see also FAQ in section 9.
- Resolution: 50 log spaced size classes, 18% bin widths.

11.4 Imaging Technology

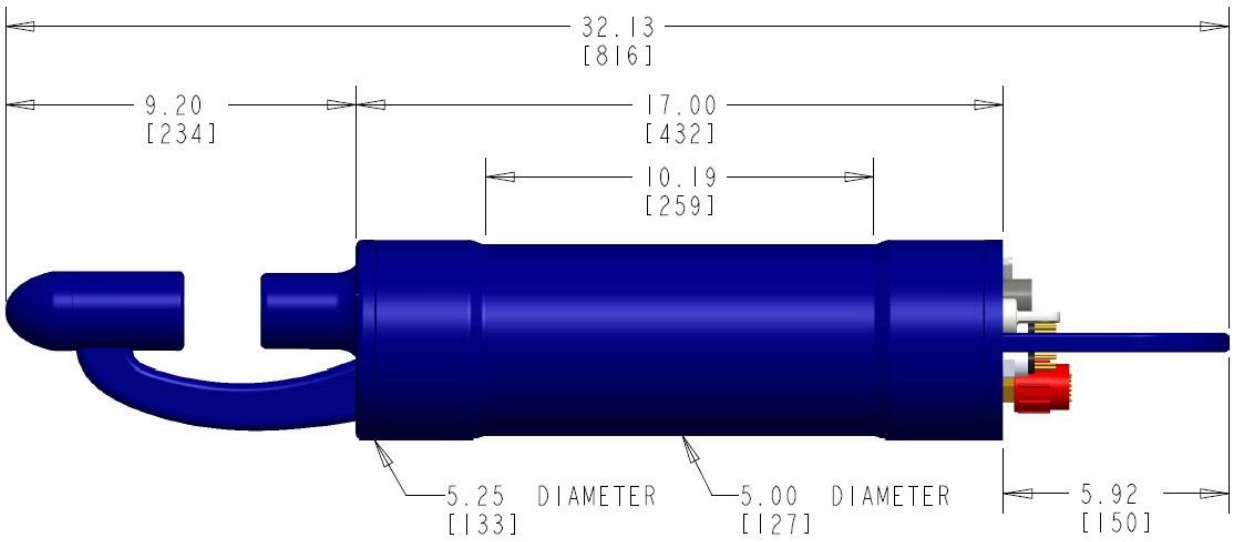
- Illumination by pulsed solid state diode laser @ 658 nm
- 4.4 µm pixel size; 1600 × 1200 pixels
- Sampling rate up to 25 holograms per second

11.5 Mechanical and Electrical

- Dimensions: 13.3 cm (5.25”) ∅ × 77 cm (30.2”) L
- Weight: 10.4 kg / 2.3 kg (23 lbs / 5 lbs) in air/water
- Maximum depth: 600 m
- 237 GB internal solid-state drive
- Battery life: 20 hours of high speed sampling for fully charged internal battery pack
- External power input: 12 to 24V @ 1.7 A maximum (18 to 24V recommended, to preserve charge in the internal batteries).

- Current consumption (current drawn from batteries or external supply, whichever is higher)
  - Sleeping: 5 mA max.
  - Waking from sleep (booting): 1.7A max for up to 40 seconds
  - Idling (web interface operating, laser off, camera off): 0.6 A typical at 12 V, 0.5 A at 18 V
  - Collecting holograms: 0.85 A average at 12 VDC input, 0.7 A at 18 V.

11.6 Dimension Drawing

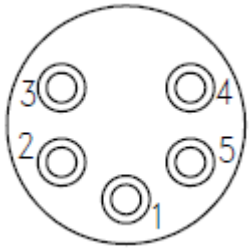


LISST-Holo2 dimensions in inches [mm]

11.7 5-Pin Bulkhead Connector Pin Assignments

Pin	Function
1	Power Ground
2	Power input, 12 to 24 V (18-24 preferred)
3	Communications Ground*
4	RS232 output, 115.2 kbaud, 8 bit, no parity*
5	RS232 input*

SubConnMCBH5P**



MALE FACE VIEW

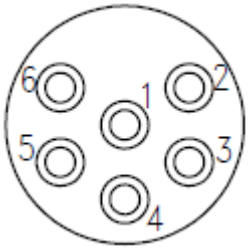
* The communications cable supplied with the LISST-Holo2 converts the RS232 signals to USB. The USB/RS232 port is used primarily for troubleshooting, not for routine operation.

** Instruments built in 2019 and earlier have Teledyne Impulse connector MCBH(WB)-5-MP-SS. Only mate with connectors from the same manufacturer.

11.8 6-Pin Bulkhead Connector Pin Assignments

Pin	Function
1	Power Ground
2	Battery Charge Input
3	Digital Output, active low
4	Digital Input, active high
5	Digital Common
6	No Connection

SubConn MCBH6M*



MALE FACE VIEW

* Instruments built in 2019 and earlier have Teledyne Impulse connector MCBH(WB)-6-MP-SS. Only mate with connectors from the same manufacturer.

12 Revision History

Version 1.1, October 2021

- Update Sequoia contact information.
- Remove warranty (for warranty terms, see www.sequoiasci.com/support/warranty).
- Remove obsolete references to 20Hz maximum frame rate (correct maximum is 25)
- Clarify connection options in section 8, Ethernet Communications.
- Clarify and correct electrical power specifications.
- Change connectors from Impulse to SubConn.
- Change “LISST-HOLO2” to “LISST-Holo2”

Version 1.0, January 2018

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