4Deep
inwater imaging

Swordfish Software User Guide

Version 1.8.2

Notices

Edition

Swordfish User Guide - Version 1.8.2

4Deep inwater imaging

6589 Chebucto Road

Halifax Nova Scotia

Canada B3L 1L9

Safety and Information Notices

Important

An “Important” signifies helpful information in using the software/hardware. It identifies an important piece of information to guide the user in their workflow, and if not followed could result in time wasted.

Caution

A “Caution” signifies a hazard. It identifies an operating procedure, or step, that if not followed precisely, could result in damage to the product or loss of information. Do not continue beyond a “Caution” sign until the procedure is fully understood.

Warning

A “Warning” signifies a hazard. It identifies an operating procedure, or step, that if not followed precisely, could result in personal injury. Do not continue beyond a “Warning” sign until the procedure is fully understood.

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1 Overview

The Swordfish software has been developed to operate in conjunction with any of 4Deep's suite of holographic microscopes (Desktop, Cuvette, Submersible [Standard 500m, 2000m and 2000m+], Autonomous).

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

The main functions of Swordfish are: to *collect* holograms (with a 4Deep microscope) and complete *sizing analysis* on groups of holograms. For viewing reconstructed holograms, and basis analysis, see 4Deep's software package Octopus. For object classification, see 4Deep's software package Stingray.

For example, Swordfish can be used for example in

- Detect and measure microorganisms such as algae or zooplankton within the water column.
- Detect particular matter and floc.
- Detect oil and gas bubbles.
- Detect particles in the industrial solutions, in food or pharmaceutical applications.

1.1 Purpose

Swordfish operates by means of recording images from the camera at the rate of up to 16 frames per second, reconstructing them and then detecting and counting particles within the volume of water. The software automatically counts, images and analyzes particles in the water from 1 micron to 2 millimeters in size. The particle size distributions can be obtained for the measured particle populations.

The measurement technique used is digital inline holographic microscopy in transmission. The objects in the holograms, imaged by the microscope, are between 1 um and 2 mm in size.

1.2 Benefits

Compared to a traditional microscope, the holographic microscope, and the Swordfish software have many benefits:

- Instant: live imaging of samples without any manipulation.
- Eliminate tasks: capable to count and measure particles automatically in 2D and 3D.
- Faster: image-based rapid particle detection up to 16 frames per second
- Larger sample volume: detect more particles than with traditional microscopy
- More control: advanced particle detection criteria, including size, intensity, threshold and shape (round & non-round)
- Better remediation: exclusively detect oil droplets and gas bubbles in water
- Versatile: open data formats allowing exchange of information
- Sample rate choices: produce particle size distributions at sample rates from 1 second to 1 hour.

2 Installation

2.1 Compatibility

The current version of Swordfish (1.8.0) is compatible to run on Microsoft Windows XP SP3 or newer.

The hologram reconstruction rate of Swordfish is up to 16 fps (2048 x 2048 pixels hologram). The particle data can be saved to a NetCDF-4 format. Analysis results are exported to MS Excel and CSV formats.

2.2 Installation Package

To install Swordfish software on your computer

- Download the software from our website: http://4-deep.com/software-downloads/.
- Insert the HASP key supplied; Follow the onscreen instructions.
- Note that for fast hologram reconstructions, 4Deep software requires a CUDA-enabled NVIDIA graphics card to be installed in the computer. For the list of CUDA-enabled graphics chips, refer to https://developer.nvidia.com/cuda-gpus.
- If your NVIDIA drivers are not up to date, please update them at http://www.nvidia.com/Download/index.aspx.
- Install Swordfish by running SwordfishInstaller.exe and following the onscreen instructions. Selecting the default parameters should typically be acceptable for most installations.

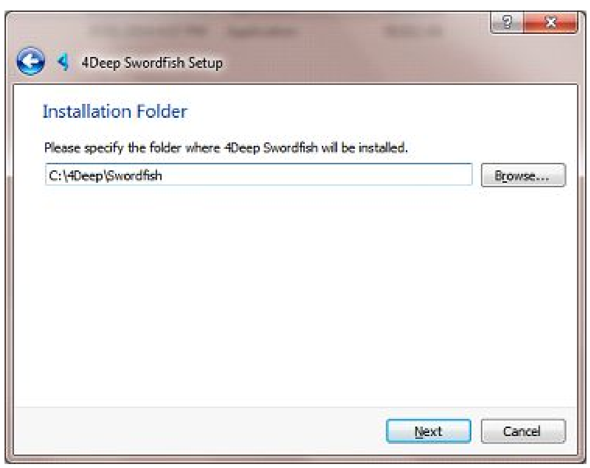

Figure 1: Swordfish installer, selection of the installation folder

2.3 Connection of the HASP dongle and starting Swordfish

After installation, attach the supplied HASP hardware protection key (dongle) to a computer USB port. Make sure the dongle light turns on. Launch Swordfish by going into **Windows Start Menu-> 4Deep-> Swordfish**. The Swordfish software will start and will automatically attempt to connect to the camera.

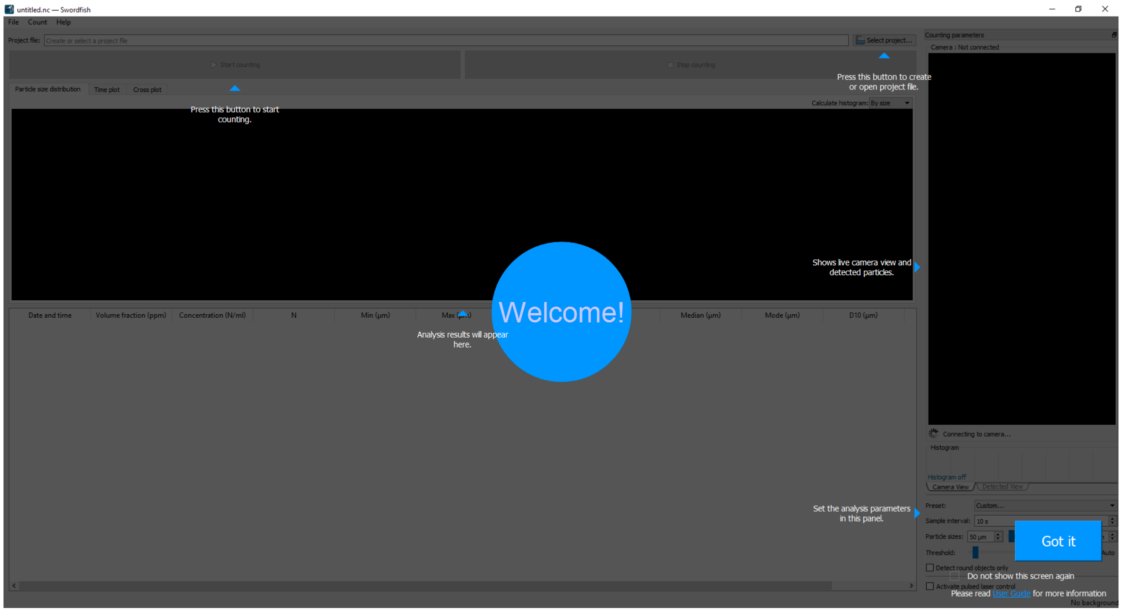

Figure 2: Swordfish software after launch

When started for the first time, Swordfish offers to select the instrument profile, or create a new one. Select the instrument profile that corresponds to the instrument being used as the instruments have different laser to camera distances and pixel sizes. Note that this dialog will only open the first time Swordfish is opened, so if you need to change these settings later they are found under Count -> Count Options (see Subsection 4.1 for more details).

Figure 3: Initial set-up of Swordfish based on the microscope used

3 System Overview

This User Guide details the functionality and use of Swordfish from a **workflow** perspective. In general, the workflow of Swordfish follows four broad categories:

- Capture/Record/Collect Holograms (Section 4)
- Object size analysis from Holograms (Section 5)
- Saving/exporting Holograms and data (Section 6)

For ease of use, the details of each Section listed above will be guided with Figures of both the Main Menu (Figure 4). The following sections briefly introduce the Home Screen of Swordfish to give users a quick overview of its layout.

3.1 Main Menu

Most of the Settings and functionality of Swordfish is located in the **Main Menu** (see Figure 4). The following gives a brief explanation of each option in the Main Menu (for more details regarding these options, see the Sections referenced).

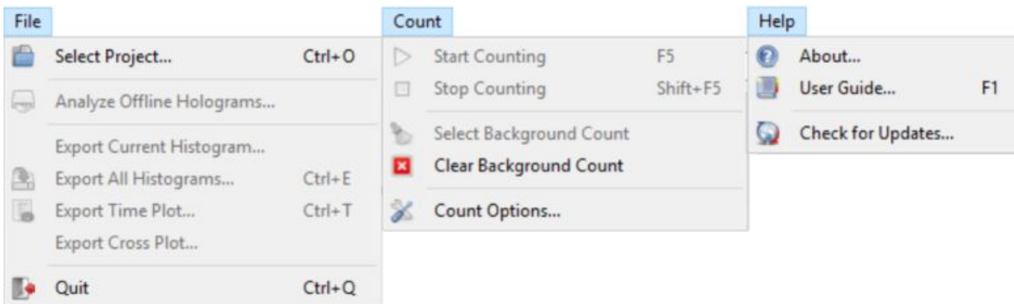

Figure 4: Main menu, showing the options under each heading

3.1.1 File

Select Project - selects the project file. Either select a new project file, or existing project file. In case the existing project is selected, the data will be appended to the file. Existing project files can be dragged to the Swordfish window and dropped in for loading.

Analyze Offline Holograms – allows for the counting of particles in previously collected holograms. Select the holograms to be analyzed from the directory, more than one at a time must be selected.

Export Current Histogram - exports current histogram and analysis data to Excel spreadsheet or CSV text file. Data for the currently selected row in the data summary table will be exported.

Export All Histograms - exports all the histograms and analysis data to Excel spreadsheet or CSV text file. Note that Excel spreadsheets are limited to 255 sheets, and therefore only 255 rows of data can be exported. There is no limitation when exporting data in CSV format.

Export Time Plot – exports the statistical data in rows of time steps to an Excel spreadsheet or CSV text file.

Export Cross-plot - exports the selected variables to an Excel spreadsheet or a CSV text file.

Quit - closes the Swordfish software.

3.1.2 Count

Start Counting - begins the counting process. Data will be appended to the current project file.

Stop Counting - stops the counting process.

Select Backgound Count - begins automated subtraction of selected count particle data from the current statistics. Refer to Section 4.4 Noise Reduction for more information.

Clear Background Count - returns Swordfish to normal statistics out put.

Count Options - opens the Count Options dialog.

3.1.3 Help

About - shows information about the software.

User Guide - opens software User Guide.

Check for updates - Swordfish will check for updates when prompted.

4 Collecting the Holograms

Important If you are analyzing already collected holograms, you should go directly to Section 5.

To collect holograms, it is important to setup both the microscope and Swordfish properly. The steps below detail how to setup and use Swordfish *in conjunction with* a 4Deep microscope. Note that you should be using both this user guide, as well as the user guide for your microscope for complete setup. You will also notice that much of the information listed below is also listed in the Desktop/Submersible User Guide.

4.1 Acquisition setup for Swordfish and the microscope

- Set up the microscope and turn it on. Please see the User Guide for the instrument you are using for setup, installation and use before continuing.
- Start Swordfish and click on the Camera Tab (right panel in Swordfish) to connect to the camera.
  - If the camera is connected, but you see a black image in the Camera View, wait 2 min for the camera auto exposure to find the correct exposure values for the current light intensity.

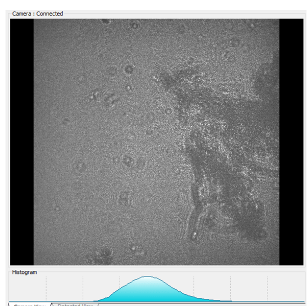

Figure 5: An example image from the camera during Swordfish operation

  - If after 2 minutes the Camera View is still black, make sure that the laser is properly connected and powered on.
  - If camera is not connected, check that the camera cables are properly attached, and power is on. Click `Connect to Camera` button to retry the connection. Note: it may take several seconds to connect to the camera. For more help on connecting the camera, see the Troubleshooting Guide (Section 7).

- The live view from the camera appear in the preview and hologram reconstructions will be performed in real time at the rate of up to 16 frames per second. Higher frame rates are possible with reduced image sizes (ex: 50 fps for 1024x1024 pixels). All reconstruction parameters can be applied to the real time reconstructions, including the reconstruction position and mode.

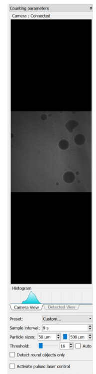

Figure 6: Camera view (top) and the camera options (bottom)

- To properly reconstruct holograms, settings need to be setup correctly in Swordfish.
  - The Count Options (under Count in the Main Menu) include:
    - **Camera pixel size** - pixel size of the camera (in μm).
    - **Laser to camera distance** - distance (in mm) between the point source and the camera sensor.
    - **Laser wavelength** - wavelength of the laser light source (in nm).
    - **Presets:** Presets are used for storing and quick recall of the optical path configurations. These can be used to store the optical configurations of the custom holographic setups, or experimental conditions. The 4 standard presets: **`Desktop`**, **`Submersible`**, **`Submersible 2000`** and **`Cuvette`** correspond to the optimal configuration of the respective 4Deep microscopes.

Caution The standard presets cannot be deleted, and any modifications to these presets will not be stored – the default values will be restored upon the preset switch.

  - For the Desktop, select the Preset Option for the Desktop: Count-> Count Options-> Optical Path ->Preset (see Main Menu Figure 4 for location). To create a custom preset, press the **`Add preset(+)`** button, and give the new preset a name. Any modifications to the preset will be stored when **`OK`** button is pressed. To delete a custom preset, press the **`Remove preset (-)`** button. The pixel size and laser wavelength settings should not need to be changed unless you are using a third-party microscope.
  - For other 4Deep microscopes, select the Preset Option for whichever microscope you are using: Settings -> Reconstruction Options-> Optical Path-> Preset.
  - For third-party microscopes, create a custom preset by pressing the **`Add preset(+)`** button, and give the new preset a name. Any modifications to the preset will be stored when **`OK`** button is pressed. To delete a custom preset, press the **`Remove preset (-)`** button.

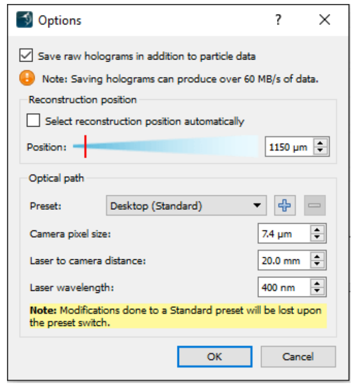

Figure 7: Select instrument preset dialog

The user can also select the plane within the reconstruction (the distance from the point source to the object within the volume) to perform counting analysis in that specific plane, using the Reconstruction plane slider, or by clicking the “Select Reconstruction Plane Automatically” option.

5 Counting and Analysis

The control of the *counting* and *recording* processes are the main function of the Swordfish user interface. The general workflow of analysis in Swordfish is:

1. Setup a Project (Subsection 5.1)
2. Setup the Parameters for Counting (Subsection 5.2)
3. Counting and Analysis (Subsection 5.3)

At this point, the microscope should be set up, with the camera connected, and the Optical Path set depending on the instrument being used.

5.1 Setup a Project

To begin analysis, the user will need to create a new project or append to a current project.

- Go to `File->Select Project...` . If you need to create a new project, type in a project name. If you are appending to an already created project, locate it in your file directory and select. With a project file selected, the program will accumulate data to the file; if an existing project is selected, Swordfish will append data to the current file. In addition, the user may choose to save raw hologram images in addition to the particle count data. To save raw holograms, go to Settings -> Count Options.

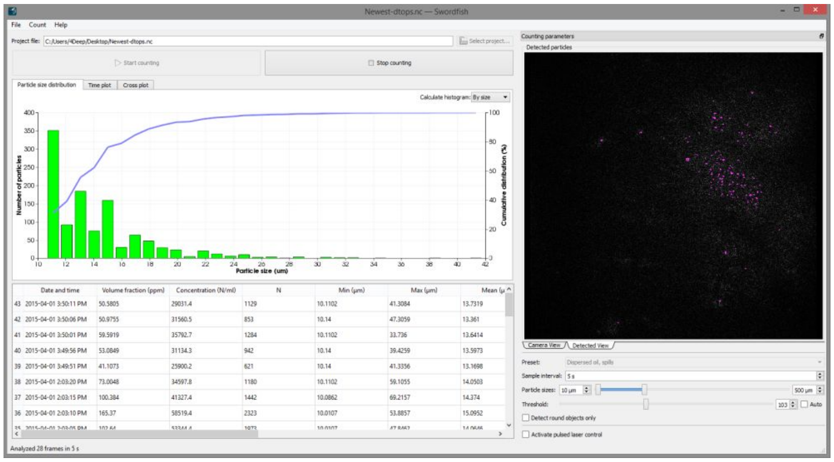

Figure 8: Example of the Swordfish interface during operation

Before the user begins a new project, s/he should know whether s/he plans on counting particles from already collected holograms (Offline holograms) or counting in real time (with the option to save the raw holograms).

- If you are analyzing offline holograms, go to `File->Analyze Offline Holograms` and select the raw hologram files you wish to analyze.
- If you are analyzing online holograms (ie counting in real-time), you also gave the option to record the raw holograms.
  - To save the raw holographic images turn the option `Save raw holograms in addition to particle data` on (`Count -> Count Options`). The images (in BMP format) will be saved into the same directory where project file is located, with the project name assigned as a prefix to the time stamp on the hologram files.

Caution Please note that a high capture rate even over a short period of time will take up much storage space on the computer. For example, sampling at a rate of 16 fps for 5 minutes will result in 19.2 GB of data (the size of one frame is 4MB). Please ensure your computer has the space requirements you need before you begin recording so as to not lose important data.

5.2 Parameters & Presets

Now that the camera and project are setup, the user will need to define and set Parameters for counting. The counting parameters will largely depend on the type of particle the user is counting. For example, if the user is testing the effectiveness of dispersants used in oil spills, the user will want to set a size range for the oil droplets, and/or use the preset for "Dispersed oil, spills".

5.2.1 Presets

There are four presets which control the counting parameters for common uses. These allow for the quick selection of the number of parameters designed for a specific application. Selecting a preset sets all the parameters (sample interval, particle size, threshold, round object detection) to the optimal values for the specific application. The user can manipulate the parameters to the specifics of the project. The custom preset is designed for the user-entered parameters and is automatically saved when the program is closed.

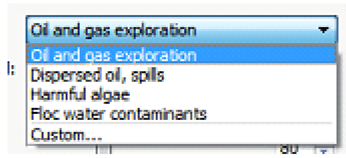

Figure 9: Preset selector

Oil & gas exploration - For the discovery of microscopic oil and gas bubbles in the water; an indicator that under water reserves may be in the vicinity. Sample interval: 10 s, Particle sizes: 60-500 μm, round object detection is on.

Dispersed oil, spills - For the detection and counting of the smaller droplets typical of dispersed oil. Sample interval: 10 s, Particle sizes: 5-60 μm, round object detection is on.

Harmful algae - For the detection and counting of potentially toxic algae. Currently the preset is designated for *Alexandrium sp*. and similar. Sample interval: 600 s, Particle sizes: 2-40 μm, round object detection is on.

Floc water contaminants - For the counting and volume fraction analysis of coagulated water contaminants in municipal systems. Sample interval: 120 s, Particle sizes: 50-500 μm, round object detection is off.

Custom - Parameters are manually set by user. The parameters set by user are remembered when Swordfish is closed and recalled on the next launch.

If the user is unsure of the conditions necessary for counting (ie exploratory analysis or unknown size range), it is strongly recommended that the user record their holograms in Octopus first. Then using the 2D Object Detection option in Octopus, the user can find optimal parameters for his/her analysis. Please see the Octopus User Guide for more options.

5.2.2 Parameters

If the presets listed above are not applicable, or the user would like to define his/her own parameters, s/he can easily do so.

First, the Sample Interval for analyze will need to be set. The Sample Interval determines the amount of time that has passed between two datasets. For example, if the Sample Interval is 10s, and the user records for 60s, there would be 6 data points in the dataset. Ultimately, this will determine the number of holograms in each data set, and hence determine the statistics of the data set. In the Main Window, the statistics for each sample period are shown. The data from each of these sample interval will represent a datum point in the other two tabs in the Main Window: Time Plot and Cross Plot.

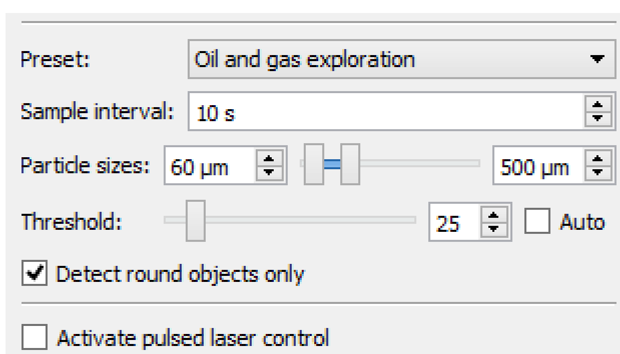

Figure 10: Swordfish particle detection parameters

Sample interval - The selection of a time frame in which the counting takes place (from 1 s to 1 h). Longer intervals give more counts, and a better particle size distribution. This setting will also determine the rate at which the histogram data is updated.

Particle size - Select the maximum and minimum size you wish Swordfish to count within the volume (from 1 to 2000 micrometers).

Threshold - The minimum intensity, or pixel value, to be considered for counting (from 0 to 255). A high threshold prevents Swordfish from counting out of focus and nondescript particles. A lower threshold can be used to count objects with high translucency. `Auto` threshold uses Otsu algorithm to set optimal threshold for particle detection automatically.

Detect round objects - If only round objects are of interest, selecting this will make Swordfish count only round objects within the volume.

To optimize the counting parameters, it is highly recommended that the user record raw holograms in Octopus first and use the 2D Object Detection to find the minimum size, maximum size and the intensity Threshold for the particular object the user is interested in. Please see the 2D Object Detection example in the Octopus User Guide for more details.

5.2.3 Pulsed Laser Control

Activate laser pulse control - The submersible microscopes from 4Deep have the ability to pulse the laser, which is controlled by the user when the option is selected. The laser will then pulse once during the exposure of the camera; in this configuration, exposure control does not change the captured hologram, only the gain and pulse duration settings will control the intensity of the hologram.

Pulsed laser control is important when the user needs to capture holograms of fast moving objects, or use the submersible microscope in flowing water conditions or towing.

Pulse duration - User control over the length of the pulse generated. Pulse durations above 6 microseconds tend to give overexposed holograms from the camera. Use a pulse duration as short as possible while still illuminating the sample; inspect the holograms from the camera feed for pixel over/under saturation; the histogram can be used as an additional visual indicator of exposure. `Auto` pulse control sets the optimal laser pulse duration/camera gain based on camera image histogram.

5.3 Counting Holograms and Analysis

At this point, all conditions to begin counting are set. Swordfish is ready to start counting.

- Press the "`Start counting`" button to initiate the particle detection and counting (also under `Count -> Start counting`).
- Press the "`Stop counting`" button to stop the particle detection and counting (also under `Count -> Stop counting`).

The particle size or volume distributions are displayed in a histogram format, and update at the user-selected sample interval. To select whether to calculate the histogram and statistics data based on particle size, select `By size` in the drop down box. To calculate histogram and statistics based on particle volume (assuming spherical particles), select `By volume` option. The scaling of the histogram axes occurs automatically for each sample interval, based on maximum particle size. The length of the sample interval determines the statistical accuracy of the particle size or volume distribution histogram.

By selecting the `D[4,3]` option, relative particle volume as a fraction of total volume of all particles (the volume in each bin divided by the total volume of all of the particles) is calculated in each histogram bin. Thus the user can identify which volume bin dominates the total volume.

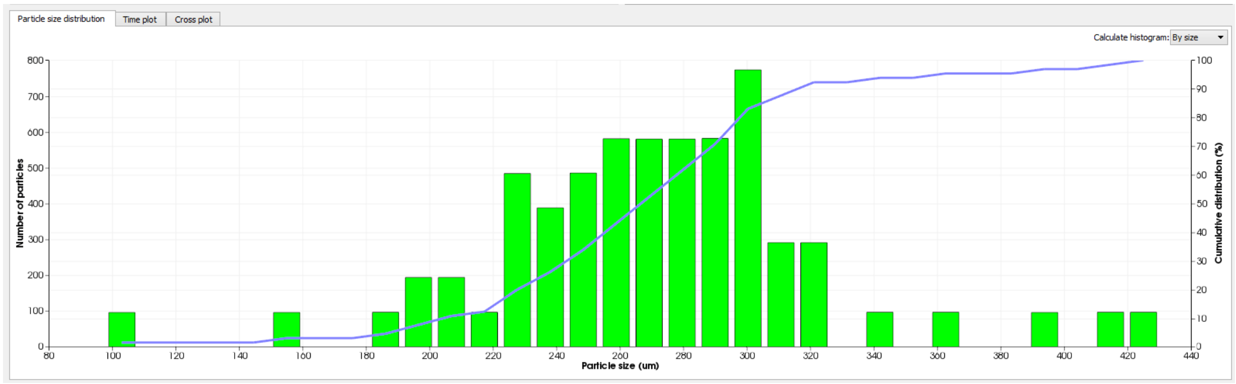

Figure 11: Particle size distribution histogram

The cumulative distribution plot (blue line) represents the cumulative particle distribution, where the total number of particles in all bins is equal to 100%, and number of particles in each bin is calculated as a percentage.

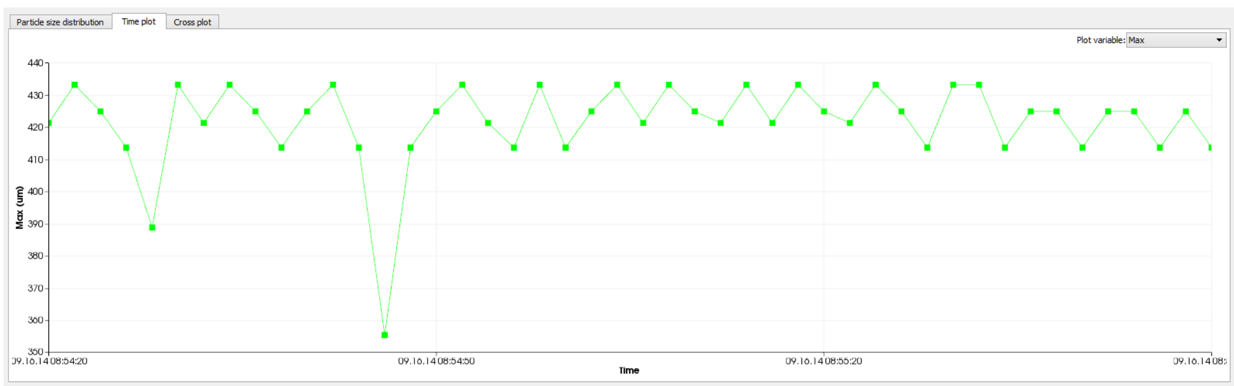

Figure 12: Particle statistics time plot

The time plots display the changes of the variable selected from the drop down menu on the right, over time. The data in the time plot is updated after each data append at the end of the sampling interval. The time plot data can be exported in MS Excel and .csv formats.

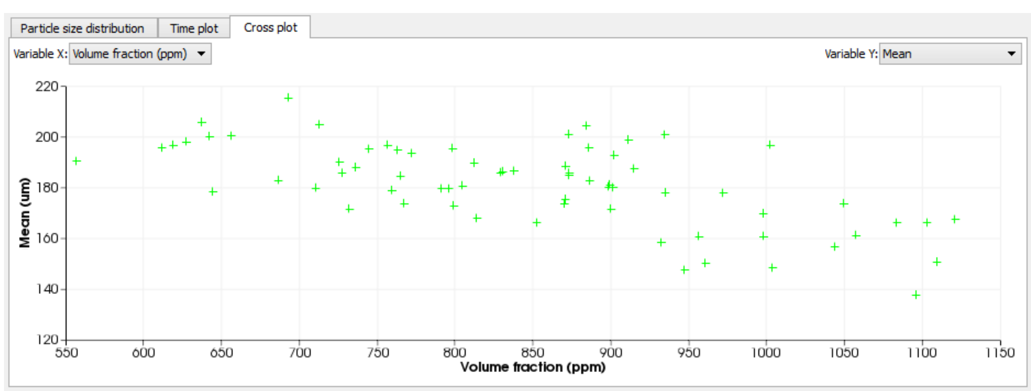

Figure 13: Particle statistics cross plot

The cross plots display the selected statistics to plot in an X-Y graph. The drop down menu in top left hand corner allows for the selection of the X variable, and the upper right one selects the Y variable. The data in the cross plot is updated after each data append at the end of the sampling interval. The cross plot data can be exported in MS Excel and .csv formats.

	Max (µm)	Mean (µm)	SD	Median (µm)	
1	238.646	141.559	37.5676	145.066	1
2	238.646	141.922	38.1789	145.304	1
3	238.646	141.109	35.6622	145.078	1
4	238.646	143.456	35.2984	145.932	1
5	236.963	143.319	35.7365	145.938	1
6	243.009	142.583	35.5027	146.158	1
7	243.009	141.57	35.6467	145.853	1

Figure 14: Data summary table

The data summary table displays the previous count data sets from the selected project file. The data displayed on each line corresponds to a single sample interval, and includes the date and time when the data was recorded, the calculated volume fraction, concentration, the measured minimum and maximum particle sizes or volumes, the calculated mean particle size or volume, median, mode, D10 and D90 of the histogram, the standard deviation, and the tallied count for the sample interval.

Increasing the sample interval will average larger amounts of data to give more accurate figures within the data summaries. The particle concentration within the volume is calculated in parts per million (ppm) taking into the account the depth of field of the imaging plane being reconstructed and background frame subtraction.

By switching the view from **`Camera view`** to **`Detected view`**, the user can then adjust parameters such that optimal object detection and counting takes place for the current conditions.

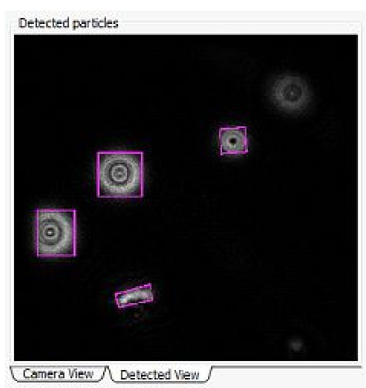

Figure 15: Detected particle view with detected particles highlighted

5.4 Use of a Background

In the instance of using Swordfish in conditions where the signal from the microscope has many particles which are not of interest to count. Swordfish can deal with this by subtracting selected data from the current out put with **`Select Background Count`**.

Record some data from the current conditions, from a sample not contaning particles of interest, and click on a out put count which well represents the background data. Select **`Count/Select Background Count`** to subtract the selected data from the incoming data of Swordfish. To return to a state where Swordfish counts all particles within the selected parameters, select **Clear Background Count**.

6 Saving Data and Images

Swordfish software can record data in formats for processing afterward. The raw particle data is saved as NetCDF-4 files. See http://www.unidata.ucar.edu/software/netcdf/ for details. NetCDF-4 files can be read out in many third party applications including MATLAB (http://www.mathworks.com/help/matlab/ref/netcdf.html) and LabView (http://zone.ni.com/devzone/cda/epd/p/id/5436).

Choose the directory to save the data by selecting the project file, and assign a new prefix to the project data if necessary. If a previous project file is selected, data will be appended to the selected file.

Figure 16: Appending particle data to the existing file

The particle size or volume distribution histogram and analysis data can be saved as MS Excel file or CSV text file. You can either export all analyzed data, or a particular row from the data summary table. To export all data, select **File/Export All Histograms...** Note that MS Excel has a limitation of maximum 255 sheets in one workbook, therefore, only maximum of 255 rows can be exported.

You can also export data associated with a particular row. Select the row in the data summary table, and then select **File/Export Current Histogram...**

The **Time plot** and **Cross plot** data can be saved to MS Excel file or CSV text file via export.

7 Troubleshooting

The most common issues, with Troubleshooting solutions listed below.

Issue	Solution
Camera not connecting in Swordfish	Check that the cables are properly attached, and power is on
Holograms are over/underexposed	Adjust Pulse duration and verify the histogram distribution (Subsection 5.2.3). For moving objects, keep exposure time as short as possible because long exposure times will result in blurry images.
Swordfish identifying and counting too many particles	Increase Threshold. Adjust particle size
HASP key is not detected	Ensure the HASP key is plugged in. Try a different USB port. Download the HASP driver from www.4-deep.com under Products -> Software Downloads

Table 1: Common troubleshooting problems and solutions

8 Appendix

8.1 Hot Key Guide

Some commands of Swordfish have hot keys for quick access.

Hot Key	Command
`Ctrl+O`	Open existing or create new project file
`Ctrl+E`	Export all histograms
`Ctrl+T`	Export time plot
`Ctrl+Q`	Quit Swordfish
`F5`	Start counting
`Shift+F5`	Stop counting
`Up arrow`	Moves the counting parameter selected up by integer values
`Down arrow`	Moves the counting parameter selected down by integer values
`Left arrow`	Moves the threshold down when selected
`Right arrow`	Moves the threshold up when selected
`F1`	Opens User Guide

8.2 Remote Control

Certain features of the Swordfish software can be controlled remotely, using the special Internet-based protocol. The remote control allows you to change Swordfish parameters from the same or different computer anywhere on the Internet. The remote control of the software operation can be performed using the supplied “Camera Remote Control” utility, or implemented in the 3d party software.

To implement the remote control in you software, you need to be able to connect, read, and write data from/to a TCP socket. The remote control is done by sending and receiving ASCII text strings through a specific TCP port. By default, all communication is happening on TCP port 1975. When Swordfish starts, it launches TCP/IP server and waits for the incoming text commands. Note that you need to use the correct IP address to connect to the TCP/IP server. If your computer has multiple network interfaces, try them all if first connection attempt fails. If you try to connect from the external network, make sure that port 1975 is forwarded by NAT to the machine that runs Swordfish on the internal network. Make sure port 1975 is not blocked by your firewall.

All remote commands have the same structure:

`COMMAND_NAME Value\n`

Where COMMAND_NAME is the name of the command to be sent/received. Value (optional) – is the value to be sent together with the command. Value is separated from the command name by a space character. Each command-value string is terminated by a new line ("\n") character.

After Swordfish software processes the incoming command, it attempts to change the respective software feature or option (for example camera recording state, or exposure value). For every valid received command, Swordfish will send a reply. Reply has the same command name as an incoming command, with "ACK_" prepended to the command name.

For example:

`FRAME_RATE 12\n`

requests Swordfish to set camera frame rate to 12 frames per second. When frame rate is successfully set, Swordfish replies with

`ACK_FRAME_RATE 12\n`

Do not assume that every command you send to Swordfish will be correctly processed. Wait for a respective ACK_ reply and take the value from the reply as a new valid value. If the value or option cannot be set, Swordfish will reply with the old valid value. For example, if we attempt to set camera burst interval to the invalid, negative value:

`BURST_INTERVAL -15\n`

Swordfish will not update the burst interval, and will reply with the current, valid interval:

`ACK_BURST_INTERVAL 60\n`

Below is the list of remote commands with short descriptions:

`ACTIVATE\n`

No values. Activates the camera.

`DEACTIVATE\n`

No values. Deactivates the camera, stops acquisition or recording.

`SYNC\n`

No values. Requests Swordfish to send current camera parameters (image directory, frame rate, burst interval, etc). All parameters will be sent as ACK_ replies. At the end of all the camera replies, ACK_SYNC reply will be sent.

`VIEW\n`

No values. Activates camera view mode. Replies with the timestamp when view mode has been activated. Timestamp is in the POSIX format – number of milliseconds since midnight, Jan 1 1970, UTC.

`RECORD\n`

No values. Activates camera record mode. Replies with the timestamp when record mode has been activated. Timestamp is in the POSIX format – number of milliseconds since midnight, Jan 1 1970, UTC.

`STOP\n`

No values. Stops the camera view/record mode. Replies with the timestamp when stop has been activated. Timestamp is in the POSIX format – number of milliseconds since midnight, Jan 1 1970, UTC.

`IMAGE_DIRECTORY Val\n`

Sets the current image directory for storing images recorded by the camera. Value is an absolute path to the valid directory where images will be stored.

`IMAGE_PREFIX Val\n`

Sets the prefix of image files. Value is a string that will be prepended to all image file names recorded by the camera.

`FRAME_RATE Val\n`

Sets camera frame rate. Value is frame rate in frames per second (floating point).

`BURST_NUMBER Val\n`

Sets camera burst frame number. Value is a number of frames in the burst of frames recorded by the camera (integer). If burst number is set to 1, continuous recording will be performed.

`BURST_INTERVAL Val\n`

Sets camera burst interval. Value is an interval in seconds between the bursts of frames recorded by the camera (integer).

`GAIN Val\n`

Sets camera gain. Value is a camera gain (usually in dB, depends on the camera model) (floating point).

`AUTO_GAIN Val\n`

Sets camera auto gain on or off. If value=0, auto gain is off, if value=1, auto gain is on (integer).

`EXPOSURE Val\n`

Sets camera exposure. Value is a camera exposure (usually in μs, depends on the camera model) (floating point).

`AUTO_EXPOSURE Val\n`

Sets camera auto exposure on or off. If value=0, auto exposure is off, if value=1, auto exposure is on (integer).

`ACTIVATE_PULSED Val\n`

Activates or disables pulsed laser mode, parameters are 0 and 1.

`STROBE Val\n`

Manual control of strobe duration, parameter is strobe duration in μs.

`AUTO_STROBE Val\n`

Parameter 1 or 0. Turns autostrobe off or on. Autostrobe is an algorithm to automatically control strobe (laser pulse) duration and gain based on histogram of received camera image.

`SAMPLE_INTERVAL Val\n`

Sample interval for data analysis (in seconds).

`ANALYZE_HOLOGRAMS Val\n`

Analyze a list of holograms offline. A list is either a list of absolute paths separated by a star(*), or a list of relative file names. Relative file names are related to the directory in which current project file (.nc) is located. Returns number of files that were not analyzed or 0 if all files have been analyzed.

`START_COUNTING Val\n`

Returns 1 if counting started and 0 otherwise; starts the counting.

`STOP_COUNTING Val\n`

Stops the counting; returns 1 if counting stopped, and 0 otherwise.

`SET_PROJECT_FILE Val\n`

Sets the name of the new project file (.nc) should be the absolute file name. Returns actual project file name. This command tries to create all directories in path if they don't exist. If project file already exists it will be loaded to append new data.

`PARTICLE_SIZE Val\n`

Sets particle size (min and max in μm) to be detected.

`THRESHOLD Val\n`

Sets threshold value from 0 - 255 integer.

`AUTO_THRESHOLD Val\n`

Sets auto threshold on or off (1 is on, 0 is off).

`ROUND_OBJECTS Val\n`

Sets detection of round objects on or off (1 is on, 0 is off).

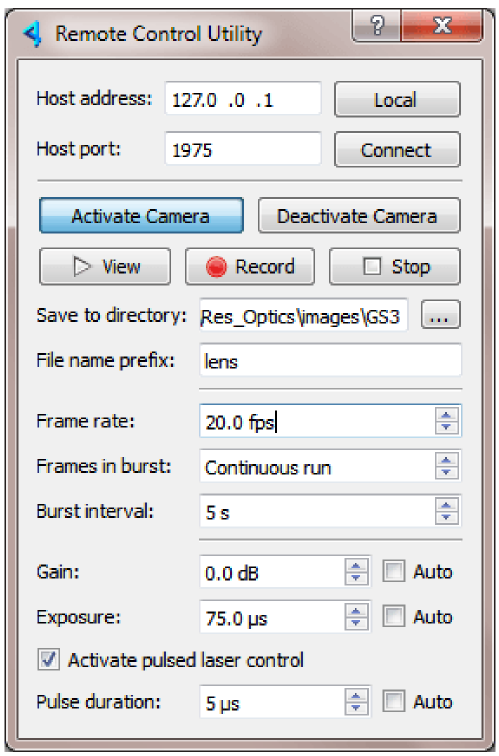

Figure 17: Camera Remote Control Utility

The convenience of Camera Remote Control utility can be downloaded and used to control Swordfish from the local or external network. The utility implements the same remote control protocol described above. Make sure you are connecting to the correct host IP address – the address of the computer that runs Swordfish software.

8.3 Principle of Operation

The Swordfish software works with the submersible or benchtop microscopes, which operate on the principles of holography to image a volume in magnification. A 405 nm laser is focused on an aperture of the same order of magnitude as the wavelength of the light, which produces a spatially coherent light source as a reference wave. Light which scatters from the objects within the media (water) will interfere with the reference wave to produce an interference pattern which contains spatial and phase information of the objects within the volume. This interference pattern, the hologram, is recorded by a CCD camera, and reconstructed mathematically to build images of the objects within the volume.

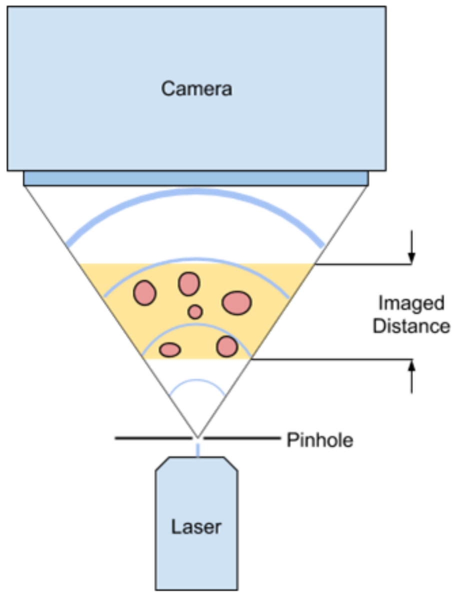

Figure 18: The basic principle of digital in-line holographic imaging

The reconstructed images can be further analyzed, saved, or assembled into the volume reconstructions based on the application. Swordfish automatically reconstructs holograms, counts and measures particles in the resulting image based on parameter settings, and develops statistics based on the measurements.

8.4 The Advantages of Holographic Microscopy

The images obtained from holographic microscopy can be compared to that of dark field microscopy, except that the collection of the images requires no constraints on the media or samples. The field of view for traditional optical microscopes using lenses is typically a few microns; with holography, a larger field of view, up to a couple of centimeters, allows for more dynamic experimental conditions.

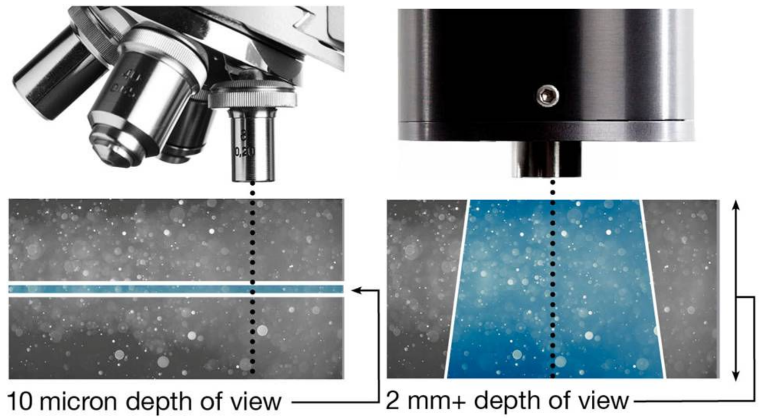

Figure 19: Field of view comparison of lens based microscope (left) and holographic microscope (right)

No need to stain or physically constrain samples with holography; the large field of view gives free movement of microscopic organisms and particles, and allows for the field deployable Submersible microscope from 4Deep, for real time, *in-situ* imaging.

Along with the spatial information providing the images in the reconstructions, holograms also contain the phase information; the differences in the speed of light passing through the objects is provided by this information, giving Quantitative Phase Imaging (QPI) capabilities to Octopus. The measurements of the thickness of objects obtained from phase information can be very precise, as the phase information exists well below the wavelength of the light.

Holographic microscopes from 4Deep work simultaneously as a QPI microscope and as a dark field microscope, and gather information from a macroscopic field of view with each frame. With frame rates of 15 fps or more, and frame exposures down to 1 microsecond on some models, the holographic microscopes of 4Deep offer 4D imaging capabilities like no other microscopes.

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