Swordfish Software User Guide

Version 1.3.0

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Introduction

The Swordfish software has been developed to operate in conjunction with either Resolution Optics Submersible or Desktop holographic microscopes. The Resolution Optics Submersible microscope is a holographic microscope which can operate while fully submerged in water. The Desktop microscope is a portable benchtop instrument.

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 either the Submersible or Desktop microscopes, please refer to the respective user guides.

Swordfish software 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.

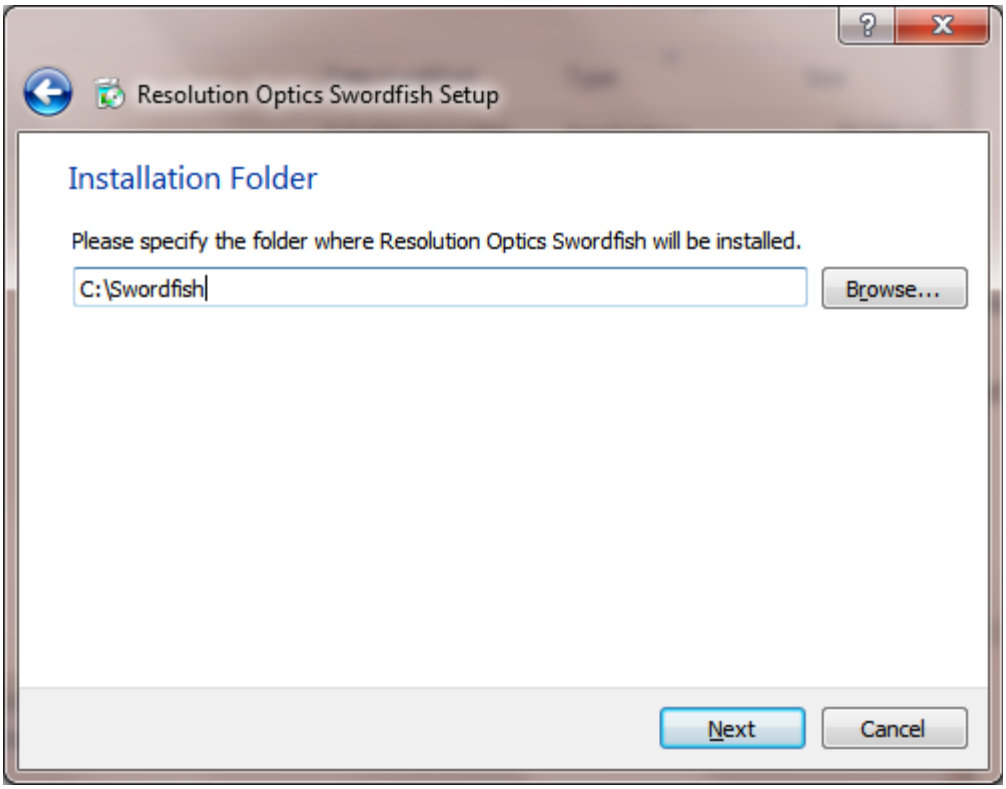
The Swordfish software can be used for example to

- 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

Quick Installation Guide

To install Swordfish software on your computer

1. Insert the installation CD, that was provided, in your computer.
2. Run HaspUserSetup.exe. Follow the onscreen instructions.
3. Run the ebus_sdk_xxx.exe, follow the onscreen instructions.
4. Install Swordfish by running SwordfishInstaller.exe and following the onscreen instructions. Selecting the default parameters should typically be acceptable for most installations.

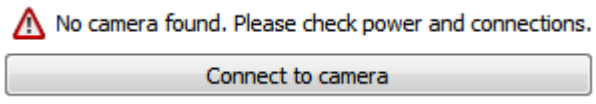


Swordfish installer, selection of the installation folder

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->Swordfish`. The Swordfish software will start and will automatically attempt to connect to the camera.

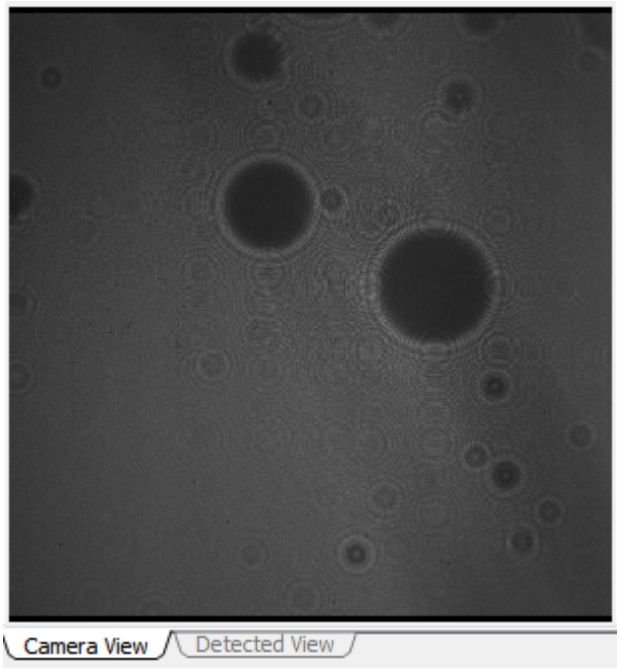
If Swordfish reports that it cannot connect to the camera, make sure that the camera cables are properly connected and that the camera is powered on. Click `Connect to Camera` button to retry the connection.



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.

If after 2 mins the Camera View is still black, make sure that the laser is properly connected and powered on.

If you see a Camera View image, you are ready to start the measurements.



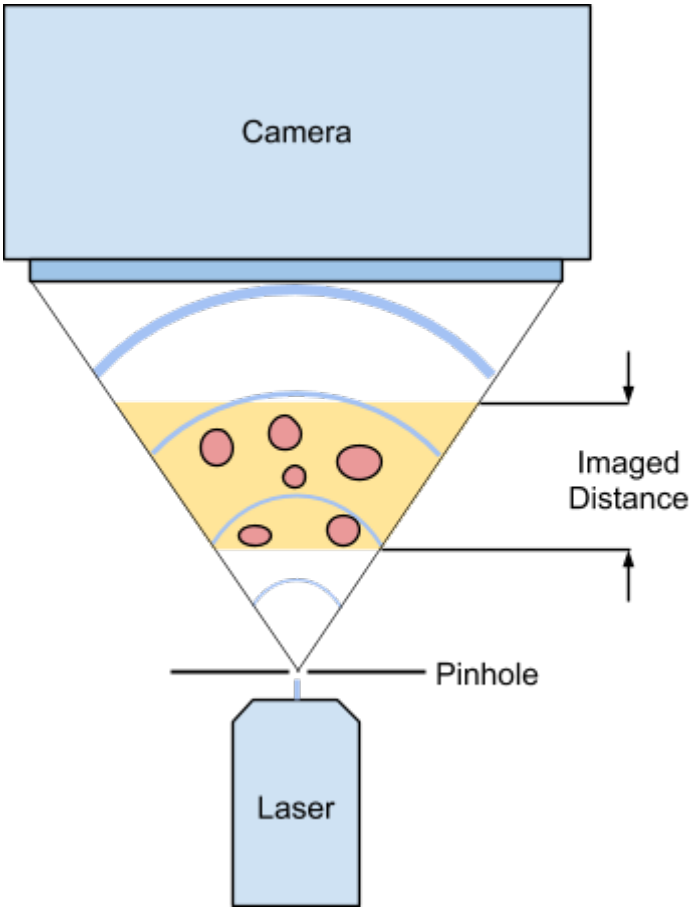
An example image from the camera during Swordfish operation

Go to `File/Select Project...` in the menu and select or create a new file for saving the particle data, and press the "`Start counting`" button to initiate the particle detection and counting.

Principle of Operation

The Swordfish software works with the Submersible or Desktop microscopes, which operate on the principles of holography to image a volume in magnification. A 405 nm laser is focused on a 500 nm pinhole 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.



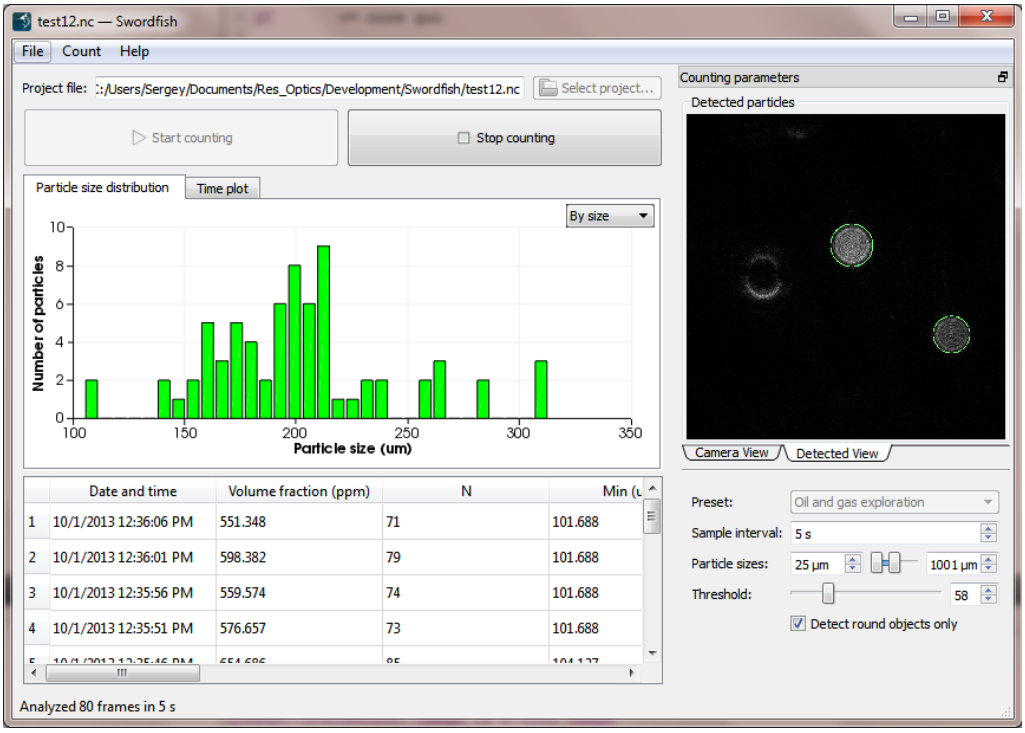
The basic principle of digital in-line holographic imaging

The reconstructed images are then analyzed and information about the detected particles is extracted from the images. The particle data is then stored into the data file and is being used to produce particle size or volume distributions, and calculate min, max and mean sizes of the particles in the population, as well as particle concentrations within the volume.

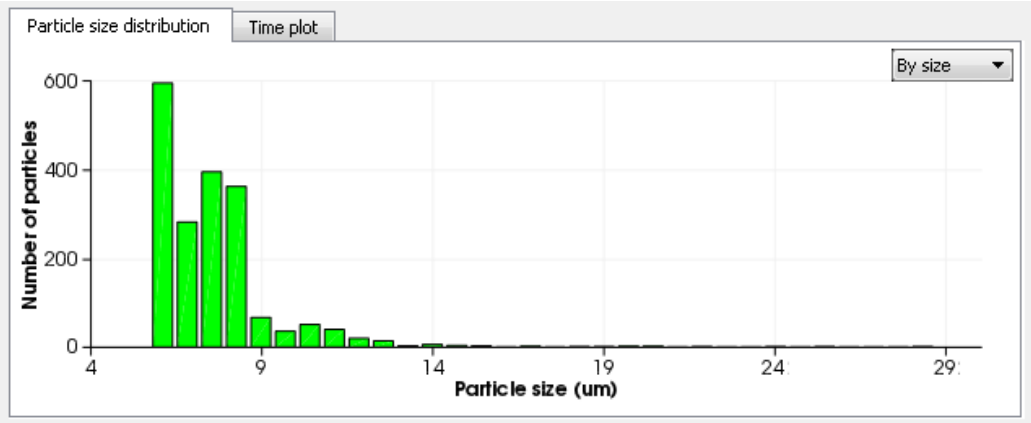
User Interface

The control of the counting and recording processes are the main function of the Swordfish user interface. 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.



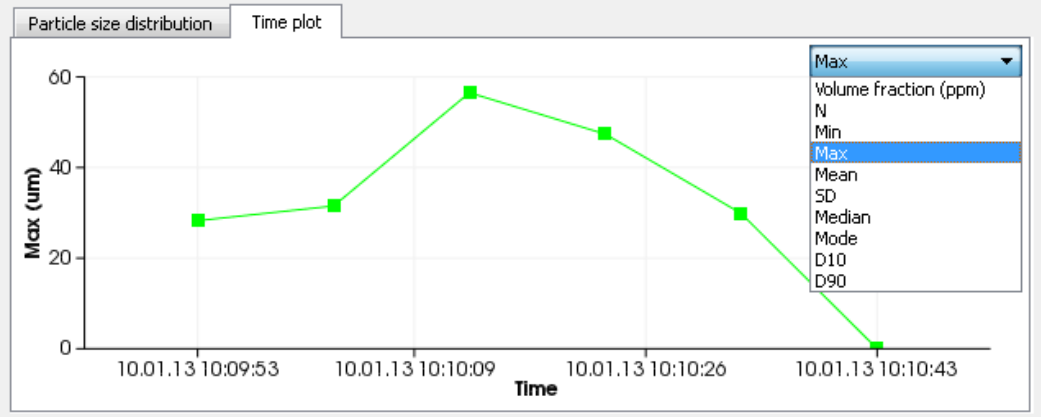
Example of the Swordfish interface during operation



Particle size distribution histogram

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. The length of the sample interval determines the statistical accuracy of the particle size or volume distribution histogram.



Particle statistics time plot

The time plots display the changes of the variable selected from the dropdown 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 format.

	Date and time	Volume fraction (ppm)	Min size (um)	Max size (um)
50	5/9/2013 10:20:29 AM	65.818	50.0328	300.245
51	5/9/2013 10:19:29 AM	56.8283	50.0542	299.764
52	5/9/2013 10:18:29 AM	168.376	52.6013	322.353
53	5/9/2013 10:17:29 AM	51.1432	50.0084	297.298
54	5/9/2013 10:16:28 AM	34.6973	52.1329	312.749
55	5/9/2013 10:15:28 AM	47.0111	51.1351	300.732
56	5/9/2013 10:14:28 AM	59.544	50.9776	299.602
57	5/9/2013 10:13:28 AM	158.172	53.6202	324.513

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, 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.

Detected particle view with detected particles highlighted

Main Menu

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.

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. 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. Note that Excel spreadsheets are limited to 255 sheets, and therefore only 255 rows of data can be exported.

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

Quit - closes the Swordfish software.

Count

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

Stop Counting - stops the counting process.

Count Options - opens the Count Options dialog.

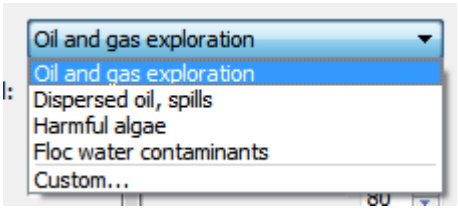
Help

About - shows information about the software.

Parameters and Presets

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.



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.

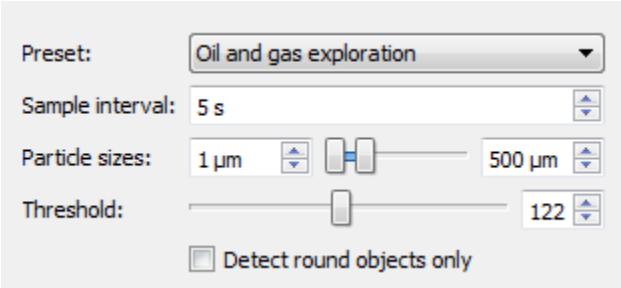
Dispersed oil, spills - For the detection and counting of the smaller droplets typical of dispersed oil. Sample interval: 10 s, Particle sizes: 5-60 µm, round object detection is on.

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.

Parameters:



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).

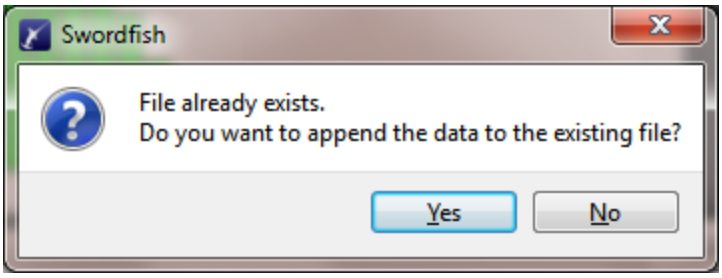
Threshold - The minimum intensity, or pixel value, to be considered for counting (from 0 to 255). A high threshold prevents Swordfish from counting out of focus and nondescript particles. A lower threshold can be used to count objects with high translucency.

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

Saving Data and Images

Swordfish software can record data in formats for processing afterwards. 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 3d 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.



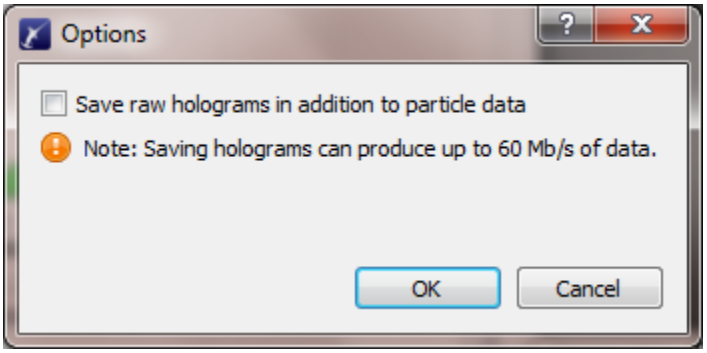
Appending particle data to the existing file

The particle size or volume distribution histogram and analysis data can be saved as MS Excel 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 data can be saved to MS Excel file.

To save the raw holographic images, go into menu `Count/Count Options...` and check the option "Save raw holograms in addition to particle data". The images (in BMP format) will be saved into the chosen directory, with the project assigned prefix.



Swordfish options dialog

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