4Deep
inwater imaging

Stingray User Guide

Version 1.4.1

Notices

Edition

Stingray User Guide - Version 1.4.1

4Deep inwater imaging

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Safety and Information Notices

Important

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

Caution

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

Warning

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

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1 Overview

The Stingray 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 Stingray software. For a detailed description of the setup and operation of your 4Deep microscope, please refer to the respective user guide.

The main function of Stingray is to classify objects according to morphology and user input. For recording, and basic analysis, see Octopus. For object identification of multiple holograms (ie particle counting), see 4Deep's software package Swordfish.

For example, Stingray can be used in:

- Marine research: algae, plankton, phytoplankton surveys
- Water quality and monitoring: microorganism imaging
- Algae production: algae profiling
- Biological research: cell biology, culture identification

1.1 Purpose

Stingray automatically finds organisms based on flexible analysis criteria, extracts morphological parameters of discovered objects, and uses powerful machine vision algorithms to identify and group objects into taxons.

1.2 Benefits

Stingray can be used to analyze offline holograms, or to obtain holograms from camera in real time to monitor aquatic environment for the specific species of interest.

- Full volume (300 slices) can be analyzed in less than 30 sec.
- Automated: Once trained, software can monitor the aquatic environment for the presence of the specific species, providing the number of organisms discovered.
- Scalable: With powerful database system incorporated, Stingray can store and retrieve hundreds of thousands of images fast. You can specify the time period of the data, or species of interest.
- Comprehensive: Over 20 morphological parameters for each discovered organism are automatically calculated and stored in the database.
- Two Operating modes:
  - Live Volume Analysis – Reconstruct, find objects in focus, extract morphological parameters, classify
  - Offline Analysis (analysis of stored holograms) : Full analysis of previously saved holograms. Change parameters to analyze different size classes
- Data grid view: Show thousands of images stored in the database
- Display morphological parameters and metadata for each object

2 Installation Guide

2.1 Installation Package

To install Stingray 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 Stingray by running StingrayInstaller.exe and following the onscreen instructions. Selecting the default parameters should typically be acceptable for most installations.

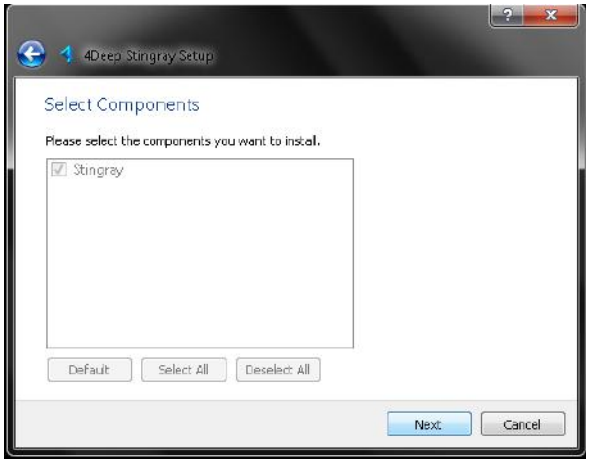


Figure 1: Stingray installer, selection of the installation package

2.2 Connection of the HASP dongle and starting Stingray

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

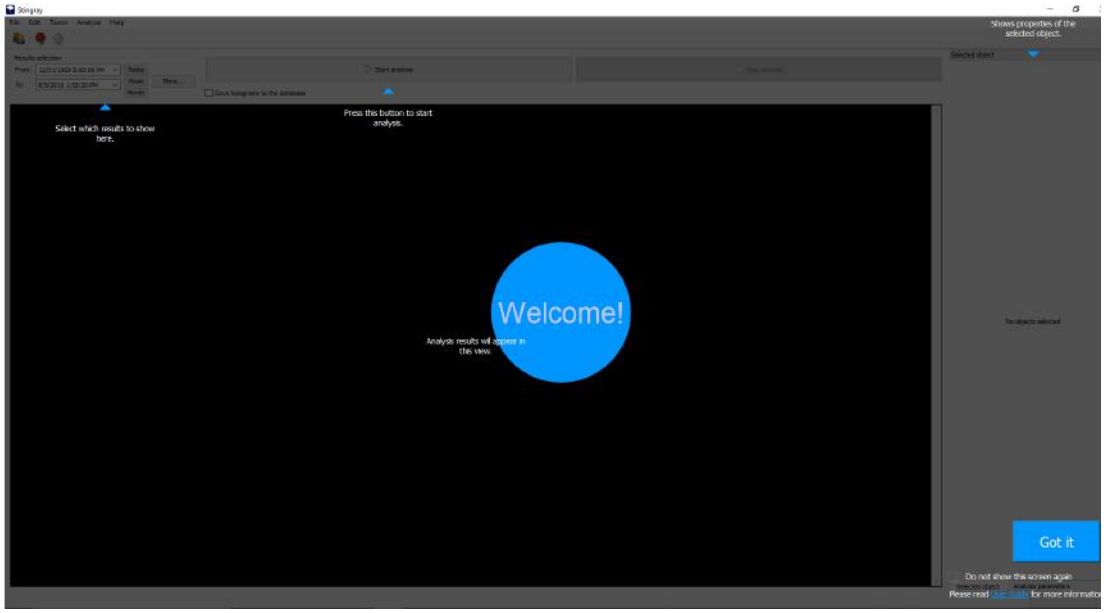


Figure 2: Stingray Software user interface after launch

When started for the first time, Stingray offers to set the recording parameters. User name, organization and location assists in the development of the database. Select the instrument profile that corresponds to the instrument being used.

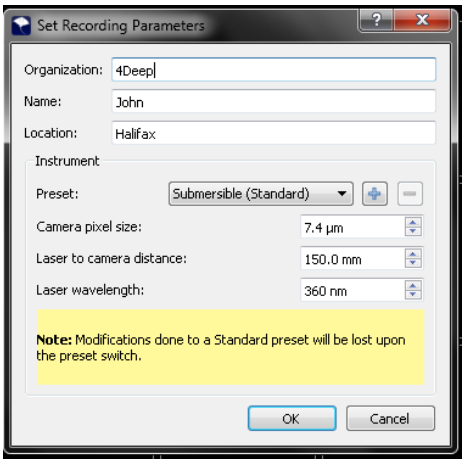


Figure 3: Set recording parameters dialog

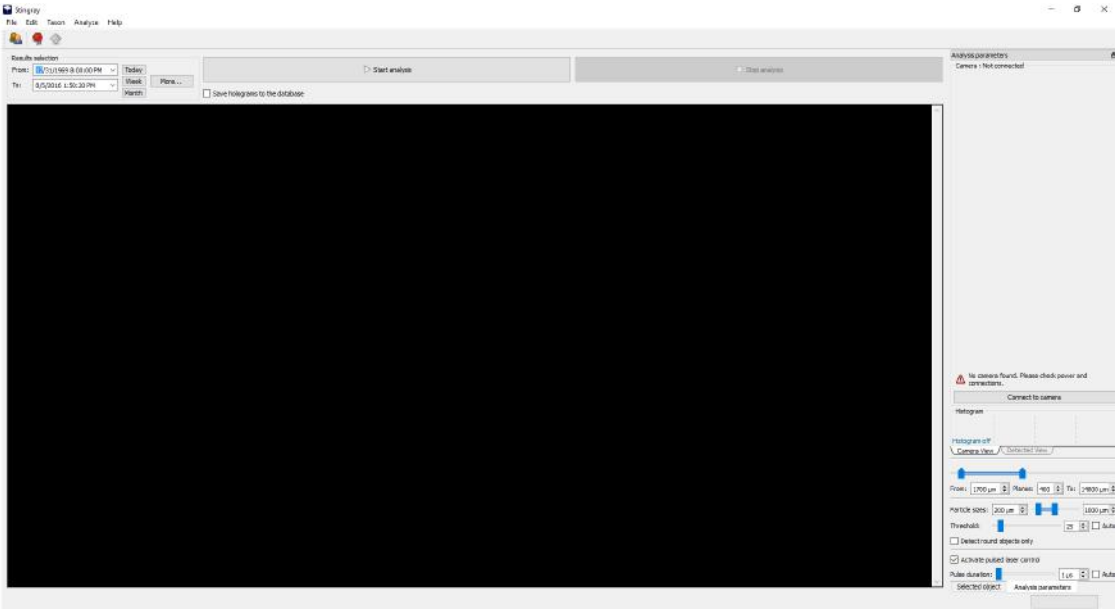


Figure 4: Stingray Software user interface after launch

3 System Overview

This User Guide details the functionality and use of Stingray from a **workflow** perspective. In general, the workflow of Stingray follows two broad categories: Analysis of Holograms and Training Classifiers.

For ease of use, the details of each Section listed above will be guided with figures of both the Main Menu (Figure 5) and Main Window (Figure 6), as well as a flow chart of the workflow (Figure 7). The following sections briefly introduce the Home Screen of Stingray to give users a quick overview of its layout.

3.1 Main Menu

Most of the settings and functionality of Stingray is located in the **Main Menu** (see Figure 5). The following gives a brief explanation of each option in the Main Menu (for more details regarding these options, see the Sections referenced).

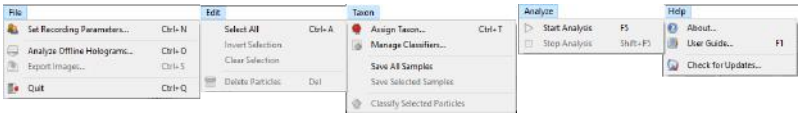


Figure 5: Main Menu, showing the options under each heading

3.1.1 File

Set Recording Parameters - This toggles the dialog where the user can set the parameters for recording. Refer to the dialog description for details.

Analyze Offline Holograms - Run Stingray on previously saved holograms. Select holograms in pairs.

Export Images - Exports selected images as PNG files.

Quit - closes the Stingray software.

3.1.2 Edit

Select All - Selects all objects identified by Stingray.

Invert Selection - Inverts the selection of objects.

Clear Selection - Clears all selected objects

Delete Particles - Deletes all selected objects.

3.1.3 Taxon

Assign Taxon - Dialog for user defined taxon assignment of selected objects. Add new taxons, or use already defined taxons.

Manage classifiers - opens the Manage classifiers window.

Save all samples - Saves all objects to the database for classifier training.

Save selected samples - Saves the selected objects to the database for classifier training.

Classify selected particles - Select and run the previously trained classifiers on the selected objects for classification.

3.1.4 Analyze

Start analysis - Starts the reconstruction and identification process on raw holograms.

Stop Analysis - Stops the running analysis.

3.1.5 Help

About - shows information about the software.

User Guide - opens software User Guide.

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

3.2 Main Window

The **Main Window** provides users with a view of the objects as they are analyzed (smaller, right panel of Figure 6), as well as a view of the classified objects (larger, left panel of Figure 6). More details regarding the functionality of the Main Window is found later, throughout the text.

Icons at the upper left toggle dialogs which are the same as those found in the drop menus (highlighted in red in Figure 6).

 **Set Recording Parameters - Assign Taxon - Classify selected particles**

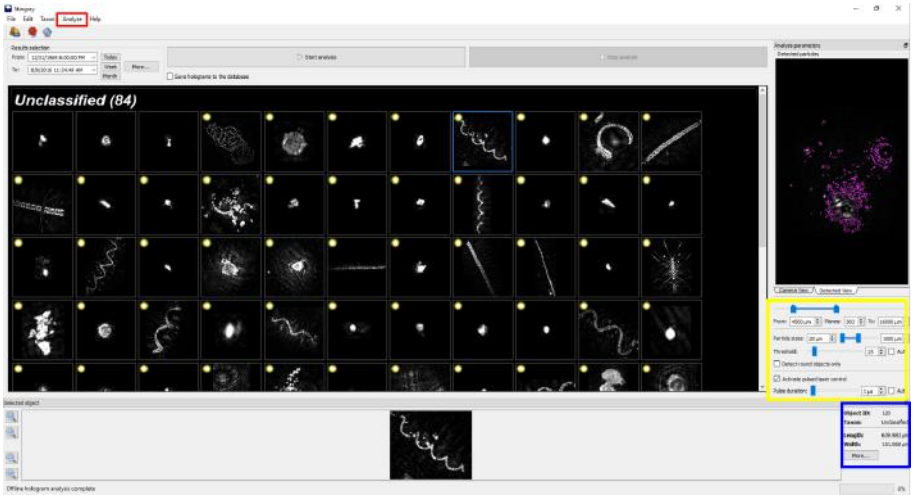


Figure 6: Main Window, with detection parameters highlighted in yellow, Analyze buttons highlighted in red, and Object Properties highlighted in blue.

3.3 Work Flow

The general workflow of Stingray follows the outline below.

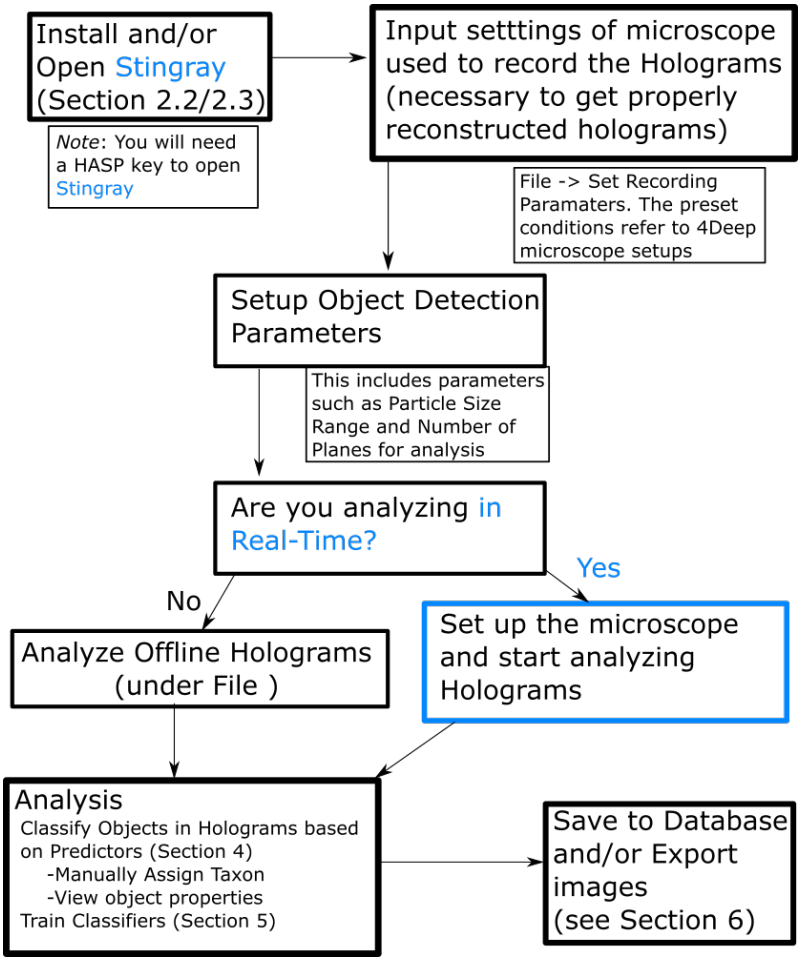


Figure 7: Flow chart of the workflow

4 Classifying Objects

Before the user can analyze holograms, s/he needs to define the hologram collection conditions (ie the microscope used for collection) as well as parameters for the classification of detected objects.

4.1 Setup

4.1.1 Recording Setup

When Stingray is first opened, the user will be prompted to input setup conditions, so if the user completed this step on startup, this step is not necessary.

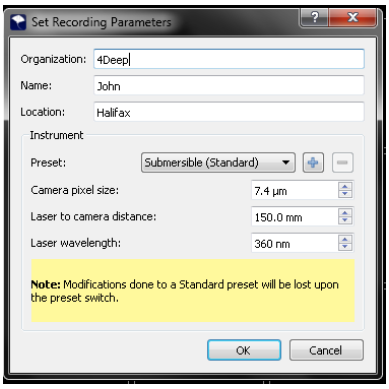


Figure 8: Set recording parameters dialog

To properly scale the holograms, **information about the microscope setup is necessary**, thus the user must input the correct microscope settings. In the Main Menu (see Figure 5), go to **`File -> Set Recording Parameters`**, and find "Instrument". Select from the **`presets`** in the drop down menu or input a customized setting.

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.

The settings are:

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.

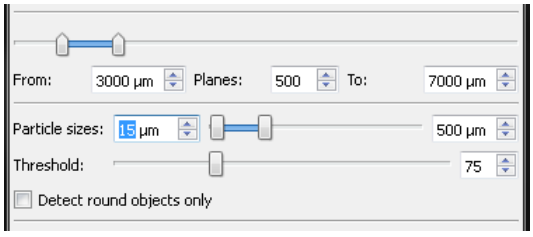
Laser wavelength - relative wavelength of the laser light source (in nm).

To create a new custom preset, change the above settings to the correct value, and press the **`Add preset (+)`** button. The name of the preset can be changed upon creation. Any modifications to the preset will be stored when **`OK`** button is pressed.

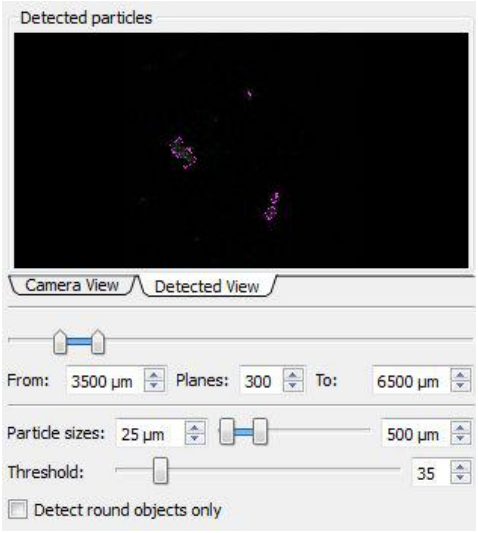
To delete a custom preset, press the **`Remove preset (-)`** button.

4.1.2 Analysis parameters

The parameters for classification setup are located in the bottom right of the Main Window (highlighted in yellow in Figure 6), and are shown below:



The **`Camera View`** tab gives the view from the camera feed of the connected microscope, and displays the raw holograms. The **`Detected View`** tab shows the the reconstruction and analysis once the analysis process is started.



The user can select the z-Axis boundaries of the volume (in μm from the point source) by using the **From** and **To** input fields. The option to select the range is particularly useful for Desktop microscope users, and those using spacers in the Submersible microscope, as the user will know the distance the sample is from the point source. The user can also change the number of **Planes**, which determines the number of reconstructions that will be performed to build the volume. The reconstructions are done at fixed intervals: (To - From) / (Number of Planes - 1).

The user should also set the **Particle sizes** (range) of objects in microns. This is useful if the user is interested in a limited size range or species.

The **Threshold** is the minimum intensity value Stingray will use to detect objects. A high threshold, for example 200, means that the intensity of the pixel has to be 200 or more for that pixel to be detected by the software. Thus, a low threshold (ex: 5) would allow many pixels to be detected, while a high threshold (ex: 200), would let less pixels be detected. Start with a low threshold and work your way up to a higher threshold. There is also an "Auto" option for threshold.

There is an option to **Detect round objects only** by checking the box (useful for oil droplet detection in the Oil & Gas industry).

Stingray also allows the user to turn on the pulsed laser, and set the pulse rate for hologram capture.

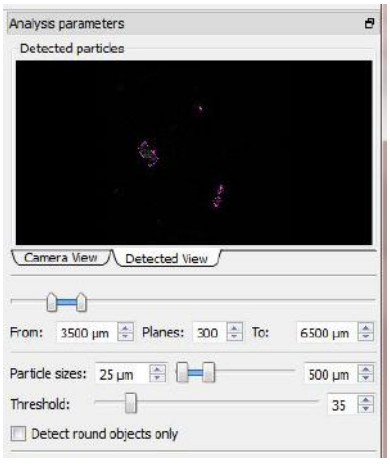


Figure 9: Control of the analysis parameters

The double window in the upper right corner,  , toggles the Analysis parameters as a separate window from the Results Display.

4.2 Analyzing Holograms

Stingray has two Operating Modes: Offline and Real-time. In both cases, the user can click the "Save holograms to database" option to save the holograms to the database.

If the user is analyzing offline holograms (already recorded), go to **`File -> Analyze Offline Holograms`**. The user will need to select at least two holograms for analysis. The benefit of analyzing offline holograms is the the flexibility of being able to re-analyze the holograms, with different settings and with different classifiers. If the user needs to record holograms, s/he should use Octopus, the general analysis software, and the Octopus User Guide.

If the user chooses to analyze holograms in Real-time, s/he needs to connect to a microscope (usually a 4Deep microscope), and should refer to the User Guide for the details of setup.

At this point, all of the setup is complete, and now the user is ready to start analyzing holograms.

4.3 Analysis of Holograms

Click the "Start Analysis" button (or **`Analyze -> Start Analysis`**). The user will be prompted by a "Select Classifier" (or can go to **`Taxon -> Classify selected particles`**).

4.3.1 Select Classifier

The user needs to select the classifying database (called a "Classifier") for selected objects. Stingray comes with some trained Classifiers, however the user can train a classifier his/herself, if desired (see Subsection).

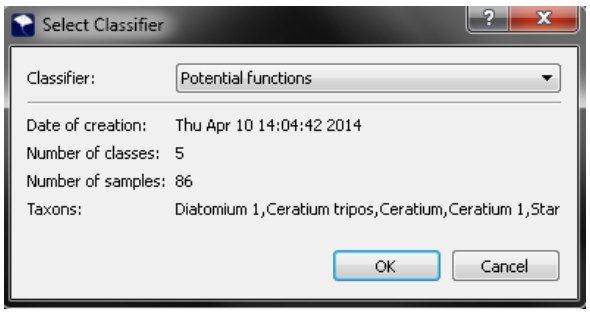


Figure 10: Select Classifier dialog

The dialog box relays information about the training of the classifier, including the date, classes, and samples, along with the taxons the classifier has been trained for. This information is valuable when you are training the classifiers, to see which classifiers work best with the selected objects.

4.3.2 Analysis

While Stingray is working, the right panel will show the user the objects being detected (based on the settings from Subsection 4.1.2) for each plane analyzed, for each hologram. Note that the analysis time is dependent on the number of objects detected (Threshold and Particle Size dependent), the number of planes selected and the number of holograms selected. To stop analysis, click the “Stop Analysis” button (or **`Analyze -> Stop Analysis`**).

4.4 Viewing Results

Once completed, the results of Stingray’s analysis process are presented in the left hand view of the Main Window. Newly detected objects will be displayed at the top of the Results Display.

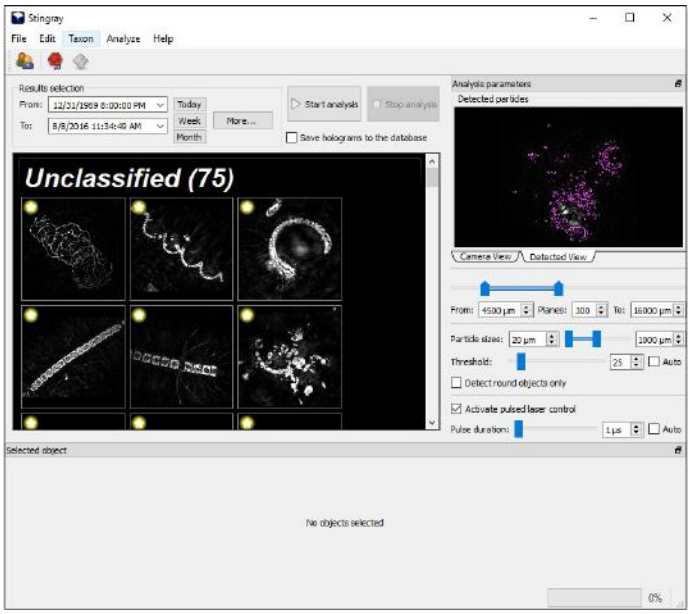


Figure 11: Display of Stingray results

To control what Results are displayed, use the **`Today`**, **`Week`** and **`Month`** buttons. These buttons select the results to be displayed, according to the date they were analyzed by Stingray. The **`From`** and **`To`** buttons toggle calendar to select a time range to display, and **`More...`** toggles the ability to select results by defined taxon.

4.4.1 Viewing Individual Object Properties

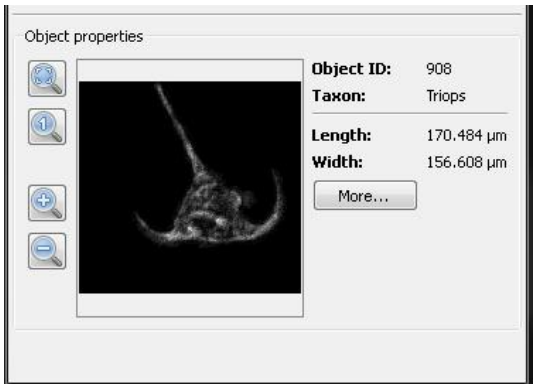


Figure 12: Selected object view and properties

When an object is selected from the results, it is displayed with its assigned properties in the bottom of the screen. Basic properties are displayed on the right, with ID, taxon, and the object dimensions. The window shows the selected objects, and the control of the reconstructed image is on the left.

 Sets the reconstruction to the Fit to window mode. The whole content of the reconstructed hologram image will be shown.

 Sets the reconstruction to the Full scale mode. The reconstructed hologram image will be scaled such that 1 pixel of the image corresponds to the 1 pixel on the screen.

 Zooms the results view in.

 Zooms the results view out.

the **More...** button toggles the Object Properties dialog.

The Object Properties dialog displays the defined object properties, and parameters for the classifiers.

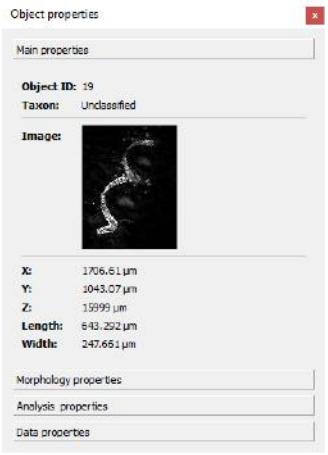


Figure 13: Object Properties dialog

Main properties - Gives the taxon, an image and ID, the position where the object was found in x-y-z coordinates, and the dimensions of the object.

Morphology properties - The morphological parameters of the selected object that are used by the classifiers.

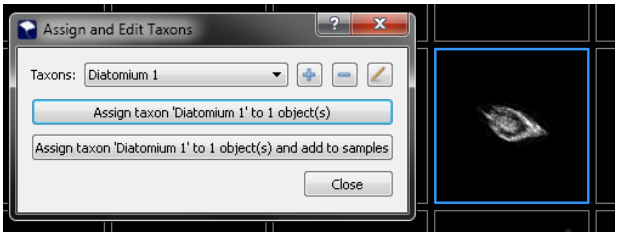
Analysis properties - A log of the user inputted properties from the Analysis parameters.

Data properties - A log of the holograms, user and instrument from which the object was extracted.

To edit multiple images at once, the user can use the options under "**`Edit ->`**" (including **`Delete Particles`**).

4.5 Assign and Edit Taxons

With new objects discovered, now the task of manual classification begins. Find good objects of known species, and select them by clicking on the images, the selections will be highlighted in blue. Selecting the Assign taxon (**`Taxon -> Assign taxon`**) will allow the user to assign the taxon, or add a new taxon, and select the image as a sample for the classifier database training.



The **`Taxons`** drop down menu gives previously identified taxon to choose from, and the user may add to, delete from or edit the list presented, with .

With a taxon selected for the new object, the **`Assign taxon '[taxa]' to [#] object(s)`** button will assign a taxa to the object, and the **`Assign taxon '[taxa]' to [#] object(s) and add to samples`** button will classify the object and put it into the database for training the automated classifiers.

Multiple quality images of the same species are necessary to add to the samples for good classification training.

5 Train classifiers

Users may find the need to train classifiers, depending on the number and diversity of the images already classified in Stingray.

- The Manage classifiers dialog (to open go to **`Taxon -> Manage Classifiers`**) is used to train classification systems to identify selected objects based on morphology parameters. Once the dialog is open, objects which are identified and selected as samples in the Results Display will be listed along with parameters of the object, including Histogram, FFT, and the other statistics the classifiers use to classify the objects. The **`Delete samples`** button will delete any selected objects from the list.

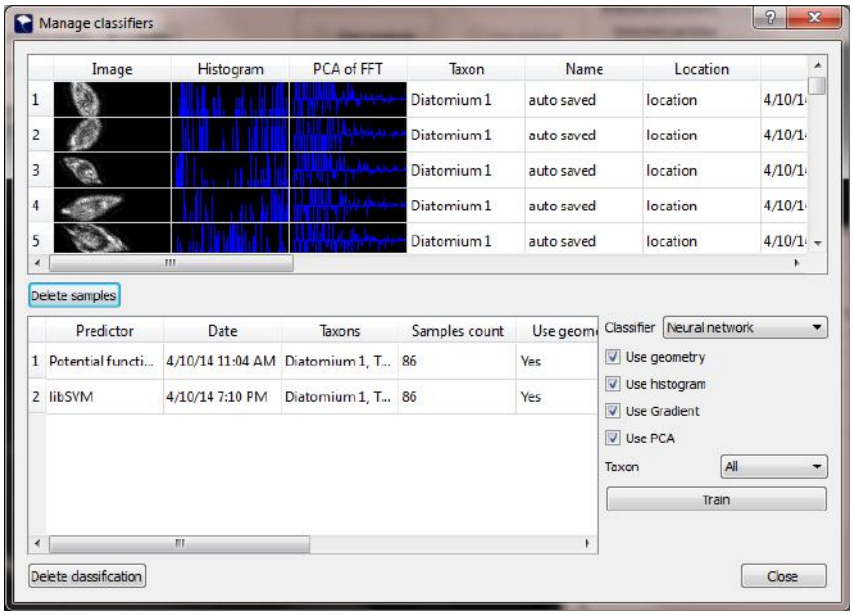


Figure 14: Manage classifiers dialog

- Choose which classifier to train, and which parameters will be used in the classification.

  Neural network - The classifier develops sets of adaptive weights which respond to parameters of the objects to be classified, and is capable of approximating non-linear functions of the parameters.

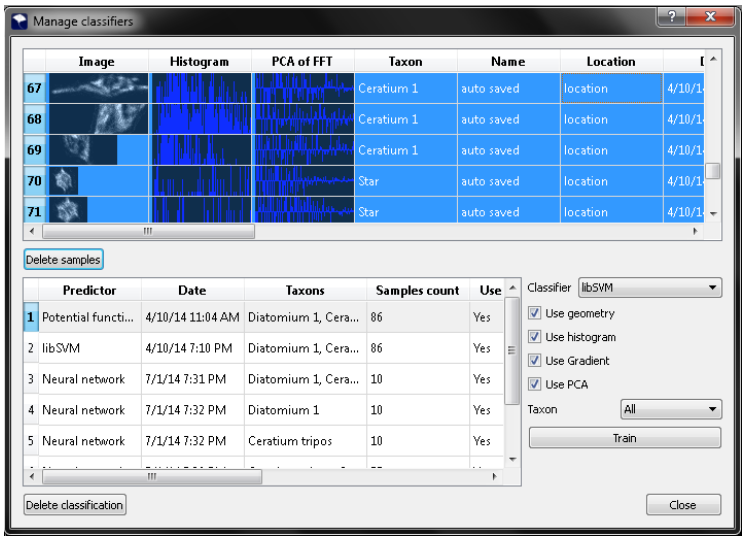
  Support vector machine - A non-probabilistic binary linear classifier which constructs additional dimension vectors to allow for easier linear separation and discrimination.

  Random forest trees - Multiple decision trees are used to output classification by the mode of the trees, using weighted nearest neighbor approaches.

  libSVM - A specific linear weighting classifier that uses a Support Vector Machine.

  Potential functions - Binary classifier that uses parameters to define identifying potential functions.

  The classifiers use different pathways to reach decisions, so one may be better than another at identifying specific objects. The user must decide which will work best for the organisms under investigation.



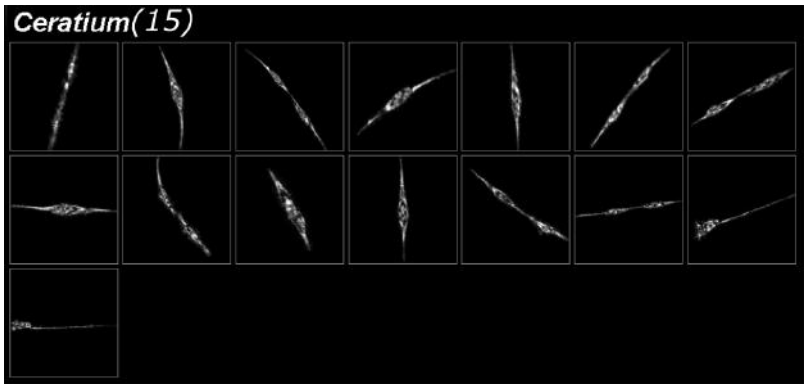
- Choose the samples to be used in the training, ensuring that at least two different taxa are selected. The **Taxon** drop down menu allows the user to select which defined taxa will be selected by the classifiers to be trained. Once all selections have been made, the **Train** button will set the classifiers to be trained using the defined parameters.
- The user can select what main parameters the classifiers will use by clicking the appropriate **Use** boxes next to **geometry**, **histogram**, **Gradient** or **PCA** of the image FFT; again, some of these will be better than others within certain classifiers to identify specific organisms. Experimentation with the ability of the trained classifiers to identify organisms correctly using the selected parameters is necessary for success.
- Run the training, using several classifiers and parameter selections, and now the classifiers are ready to be tested on the images.
- Once trained, the classifier is added to the list on the lower left. The list includes all of the selected parameters for training the classifier, and can be used in deciding the quality of the classifier for the organisms under investigation. If the classifier is unsatisfactory to the user's needs, the **Delete classification** button will remove any selected classifiers from the list.
- The satisfactory classifiers can now be used on selected particles with the **Classify selected particles** command in the Results Display.

5.1 Run classifiers on samples

Once the classifier is trained, the user will need to run samples with the classifier to ensure that it is successful.

Select the images in the Results Display you want the classifier to classify. From **Taxon -> Classify selected particles** the selection dialog is toggled.

Run the selected classifier on the images, and Stingray will classify the particles it identifies, and add them to the appropriate taxon categories in the Results Display.



If the trained classifier does not produce good results, then the classifier may be discarded in the Manage classifiers dialog.

Important *If this is the case, then training must be done again*. The type of classifier to be trained can be changed, or the parameters used in training the classifier. The quality and amount of images selected for training samples can be made to increase the ability of the classifier to be trained on specific organisms.

Repeat the tests with the newly trained classifiers, and if results from the classification are satisfactory, the classifiers are ready to use on more holograms.

With functioning trained classifiers, Stingray can now operate as an automated morphology detection and classification program.

6 Saving

When completed, the user can save all of the samples or a selection of samples (**`Taxon -> Save all samples`** or **`Taxon -> Save selected samples`**) to the database.

The user can also save the objects as images (**`File -> Export images`**).

7 Troubleshooting

The most common issues, with Troubleshooting solutions listed below.

Issue	Solution
Camera not connecting in Stingray	Check that the cables are properly attached, and power is on If there are more than one Ethernet ports on the computer, try a different one If you are using a laptop computer docking station, use the Ether net port on the docking station (not the laptop itself)
Point source not producing much light	Background light must be reduced to obtain optimal images. Try using a light shield (standard with Desktop microscope)
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 Remote Control

Certain features of the Stingray software can be controlled remotely, using the special Internet-based protocol. The remote control allows you to change Stingray 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 Stingray 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 Stingray 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 Stingray 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, Stingray 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 Stingray to set camera frame rate to 12 frames per second. When frame rate is successfully set, Stingray replies with

```
ACK_FRAME_RATE 12\n
```

Do not assume that every command you send to Stingray 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, Stingray 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
```

Stingray 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 Stingray 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).

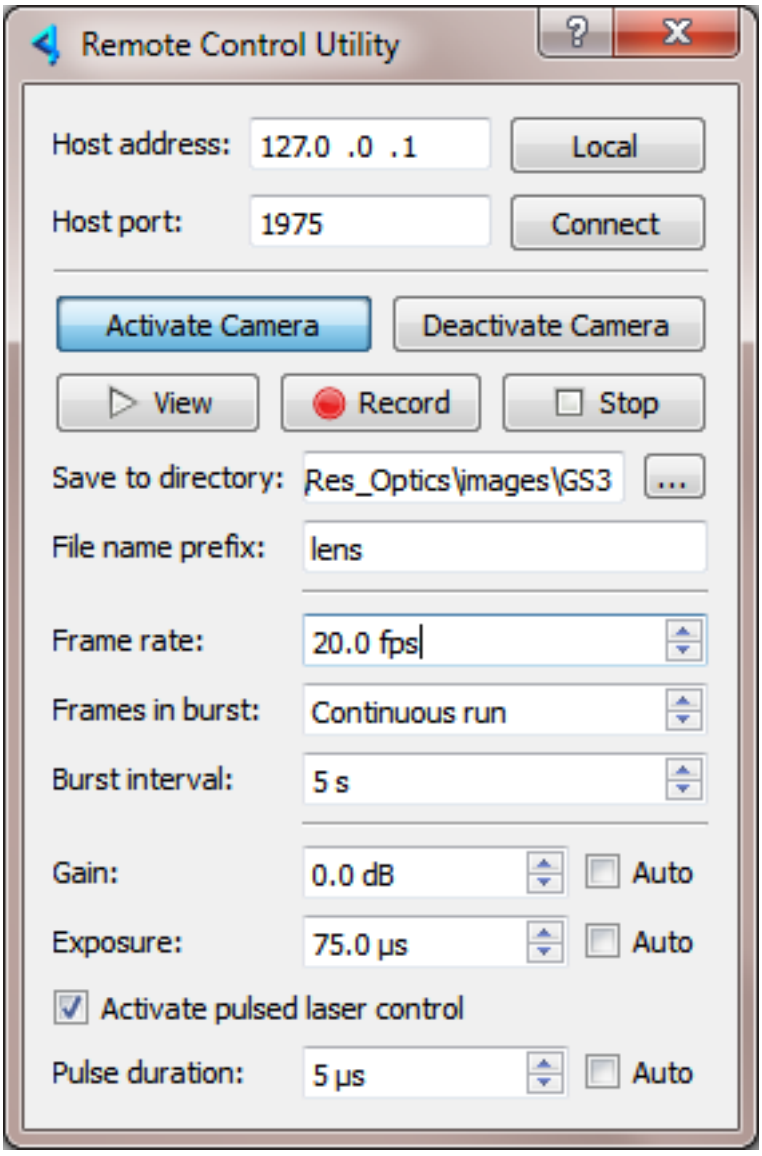


Figure 15: Camera Remote Control Utility

The convenience of Camera Remote Control utility can be downloaded and used to control Stingray 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 Stingray software.

8.2 Principle of Operation

The Stingray 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.

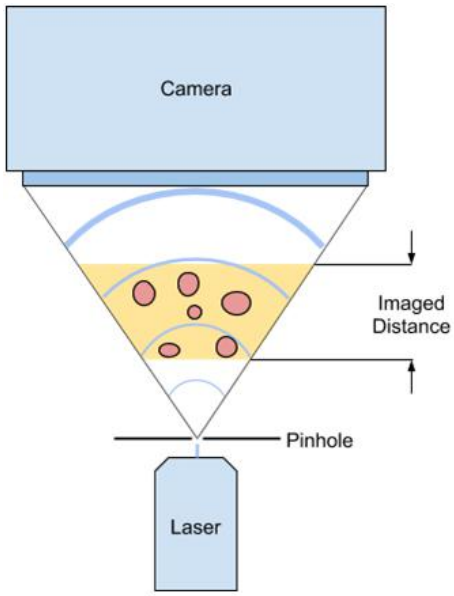


Figure 16: The basic principle of digital in-line holographic imaging

The holograms can be further analyzed, saved, or assembled into the volume reconstructions based on the application.