4Deep
inwater imaging

Stingray User Guide

Version 2.0.0

Notices

Edition

Stingray User Guide - Version 2.0.0

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+]).

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.

There are two main functions of Stingray:

1. Find objects from holograms, assigning morphological properties to the object
2. Replace with Classify objects using deep neural network image classifier

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 taxa.

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 seconds
- 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 Computer requirements

- CPU Core i5 or i7
- 8 GB of RAM
- 256 GB or larger SSD Drive
  - The microscope can records at sub-second speeds (15 fps for the S5 Submersible and 22 fps for the S6 Submersible), and each image is 4 MB, leading to a large database, thus storage may be an issue
- NVIDIA GeForce or Quadro GPU with at least 2 GB of graphics RAM, Kepler architecture or later
  - 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
- Gigabit Ethernet port (for data connection)
- 1 USB port for the HASP key
  - Full versions of the software require a valid HASP key (dongle) to run
  - Demo versions can be used without a HASP key
- Windows 7 or 10, 64-bit
  - Windows 10 Pro is preferred over other versions (such as Home)

2.2 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, shown in Figure 1.

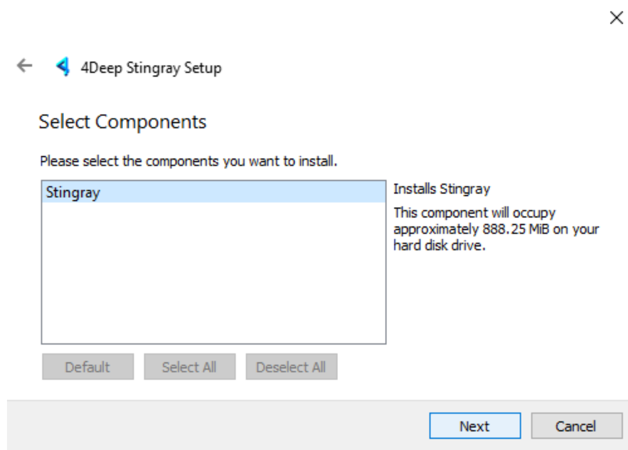


Figure 1: Stingray installer, selection of the installation package

2.3 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, and appear as Figure 2.

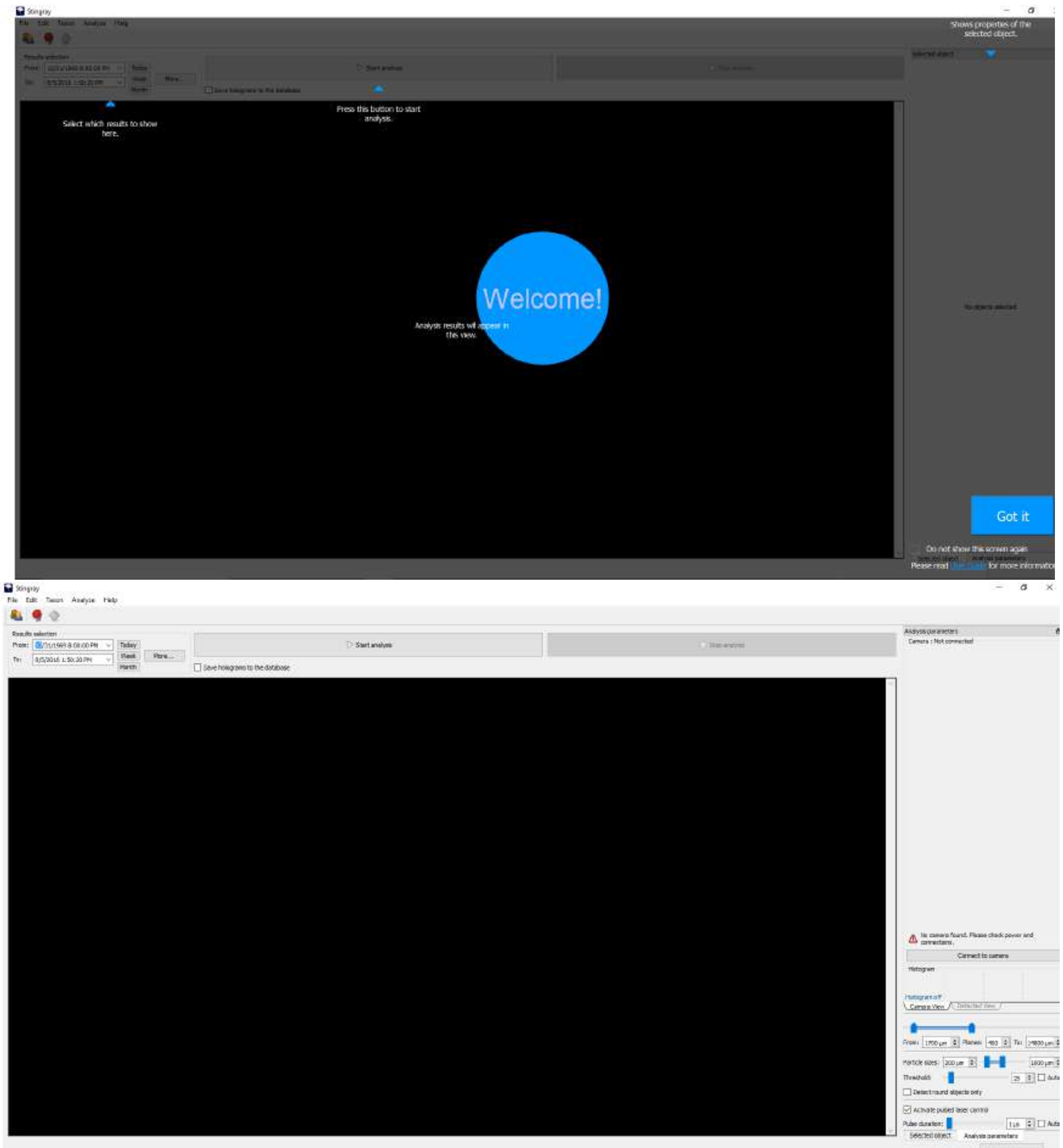


Figure 2: Stingray Software user interface after launch, with (top) and without (bottom) the initializing screen

The initial screen can be turned off in future opens of Stingray, by clicking on “Do not show this screen again”.

When started for the first time, Stingray offers to select the instrument profile, or create a new one. User name, organization and location assists in the development of the database. Click the preset in the drop down menu that corresponds to the instrument being used (typically the one you purchased), as the instruments have different laser to camera distances and pixel sizes. Note that this dialog will only open the first time Stingray is opened, so if you need to change these settings later they are found under **`File-> Set Recording Parameters`** for more details, as shown in Figure 3). Unless you have multiple microscopes from 4Deep, this setting will never need to be changed.

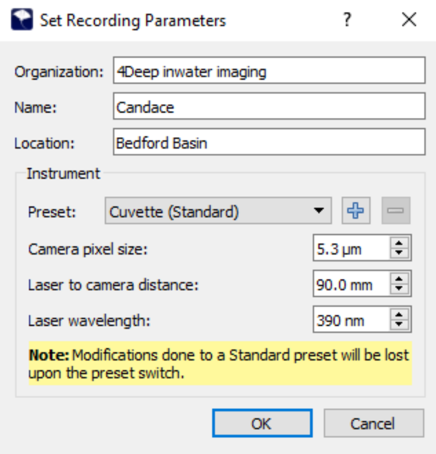


Figure 3: Set recording parameters dialog

Caution If the user chooses to record holograms as well (see Section 6), Stingray will embed the information from the instrument profile into the holograms as metadata. It is essential the user inputs the correct profile before recording so that the data embedded in the holograms are correct. These data attached to the hologram will be used by 4Deep software when the holograms later used. If you accidentally record holograms with incorrect settings, please contact a 4Deep representative for troubleshooting advice, as you may lose information from the hologram with incorrect settings.

3 System Overview

This User Guide details the functionality and use of Stingray from a **workflow** perspective. In general, the workflow of Stingray follows three broad categories: Find objects from holograms, Classify objects into Taxon and Train Classifiers.

For ease of use, the details of each Section listed above will be guided with figures of both the Main Menu (Figure 4) and Main Window (Figure 5), as well as a flow chart of the workflow (Figure 6). 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 4). The following gives a brief explanation of each option in the Main Menu (for more details regarding these options, see the Sections referenced).

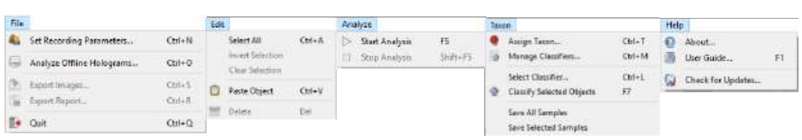


Figure 4: Main Menu, showing the options under each heading

3.1.1 File

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.

Export Report- Exports an Excel spreadsheet of the objects in the database that are selected.

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

Paste Object - pastes an object that has been copied from Octopus.

Delete - Deletes all selected objects.

3.1.3 Analyze

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

Stop Analysis - Stops the running analysis.

3.1.4 Taxon

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

Manage Classifiers - opens the Manage classifiers window.

Select Classifier - opens dialog to allow the user to change classifiers if there are more than one. Or can open the dialog “Ctrl + L”.

Classify Selected Objects - Select and run a previously trained classifier on the selected objects for classification.

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.

3.1.5 Help

About - shows information about the software.

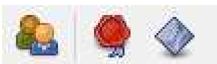
User Guide - opens software User Guide.

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 5), as well as a view of the classified objects (larger, left panel of Figure 5). More details regarding the functionality of the Main Window is found later, throughout the text. Note that when Stingray is first initialized, the Database will not have any objects. The objects will only be added once holograms are analyzed.

Icons at the upper left toggle dialogs which are the same as those found in the drop menus.

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

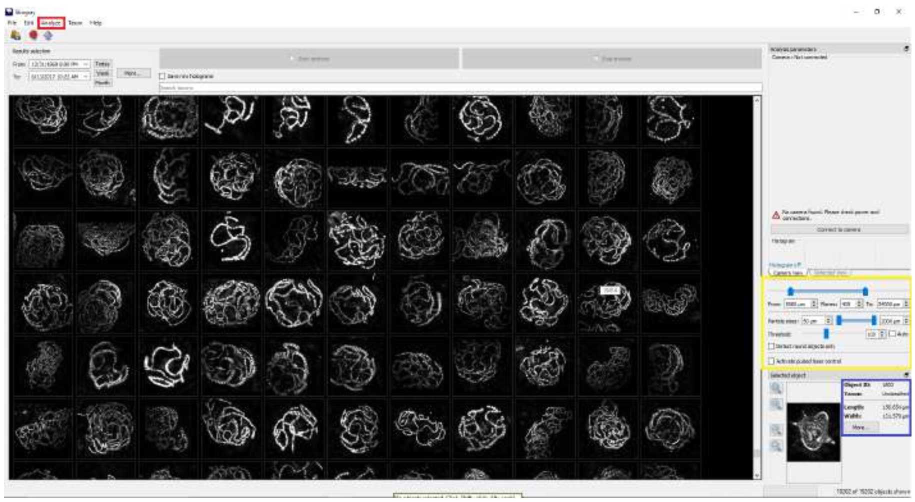


Figure 5: Main Window, with detection parameters highlighted in yellow, Analyze buttons highlighted in red, and Object Properties highlighted in blue.

3.3 Work Flow

The general workflow of Stingray follows the outline below.

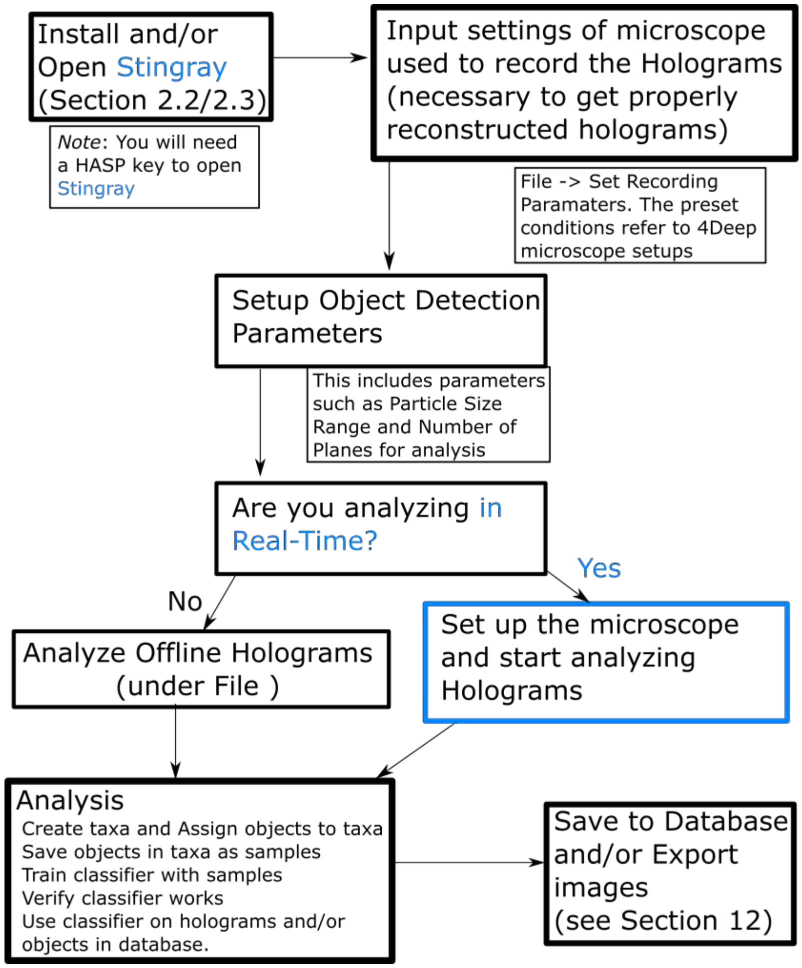


Figure 6: Flow chart of the workflow

4 Analysis

The main functions of Stingray include finding objects, classifying objects and training classifiers.

In practice, the user will need to train their own classifier(s) based on their own samples or data, as the presence of species is often dependent on geography.

Below is a list of steps the user will take to use Stingray:

1. Setup (Section 5)
   (a) Recording Setup (Subsection 5.1)
   (b) Analysis Parameters (Subsection 5.2)
2. FIND objects (Section 6)

3. Create, Assign and Edit Taxon (Section 7)
4. Save Objects to Sample (Section 8)
5. Train Classifiers (Section 9)
   (a) Select Samples to Train Classifiers (Subsection 9.1)
   (b) Train Classifiers (Subsection 9.2)
6. Run Classifier on Objects (Section 10)

5 Setup

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.

5.1 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.

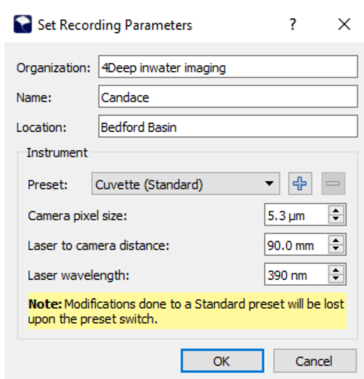


Figure 7: Set recording parameters dialog

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 4), go to `File -> Set Recording Parameters`, and find the "Instrument" section. Select from the `presets` in the drop down menu or input a customized setting.

The 4 standard presets: `Desktop (Standard)`, `Submersible S6 (Standard)`, `Submersible S5 (Standard)` and `Cuvette (Standard)` 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:

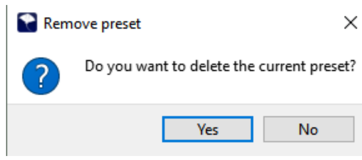
Camera pixel size - pixel size of the camera (in μm).

Laser to camera distance - distance (in mm) between the point source and the camera sensor.

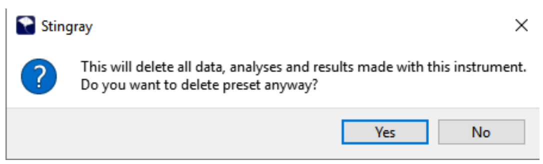
Laser wavelength - relative wavelength of the laser light source (in nm).

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. When deleting an Instrument profile, the user will be prompted to verify the deletion.

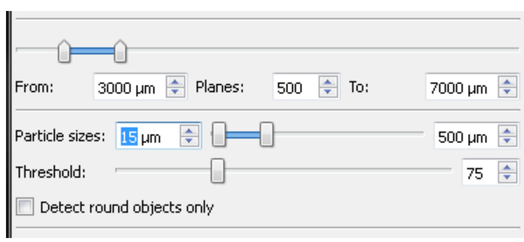


Caution The user will be prompted a second time to verify the deletion of the Instrument profile. When an Instrument profile is deleted (only the non-standard profiles can be deleted) all of the objects recorded on that Instrument profile will also be deleted. Be very cautious with deleting the Instrument profile, as the user may permanently delete objects in the database.



5.2 Analysis Parameters

The parameters for classification setup are located in the bottom right of the Main Window (highlighted in yellow in Figure 5), 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 (shown in Figure 8) shows the the reconstruction and analysis once the analysis process is started.

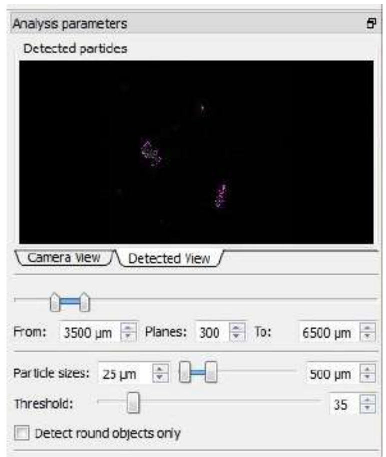


Figure 8: Control of the analysis parameters

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. Typically, for the Submersible microscope, the **From** will be between 5000-8000μm, and the **To** will range between 20000-24000μm. The user can also change the number of **Planes**, which determines the number of reconstructions that will be performed to build the volume. Typically, the number of planes will be between 100-400, depending on both the **From** and **To** range, as well as the size of the particles in the sample. Note that the reconstructions are done at fixed intervals: (To - From) / (Number of Planes - 1). When a small number of planes are used, in a large volume, for example, 11 planes from 5000μm to 15000μm, then the planes are being reconstructed every 1000μm. In this case, small objects, for example 50μm in size, could be missed, by “jumping” over the particle.

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. Note that the minimum is limited to 20μm and the maximum is 2000μm.

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 (for example 30) 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 the detection of round algae cells in ocean science, or 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, using the **Pulse duration**.

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

6 Find Objects

Once the user has setup the Analysis Parameters, s/he will need to decide the Operating Mode to run Stingray.

Stingray has two Operating Modes: Offline and Real-time.

In Offline mode, Stingray runs analysis on holograms already collected, usually pre-recorded with Octopus. 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.

In Real-time mode, Stingray runs analysis on holograms being collected by the camera. Note that in Real-time mode, Stingray does not process holograms at the frame rate, but rather, at the rate Stingray can process one pair of holograms at a time, which usually takes around 30 seconds or more. One other option in Real-time mode is that the user can click the **Save holograms to database** option to save the holograms to the database, for future analysis. 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.

6.1 Analysis of Holograms

Stingray can operate in real-time or offline modes.

Initially, there are no classifiers in Stingray, by default. Before analysis begins, the user will be prompted to use a classifier. If there are no classifiers, or if the user does not want to use the classifier, select **DON'T CLASSIFY**. If **DON'T CLASSIFY** is selected, Stingray will find and classify all objects it finds as “Unclassified”. If a classifier is used, Stingray will classify all objects it finds using the Taxon in the classifier, as well as using the “Unclassified” category if necessary the object does not fit any of the trained taxa.

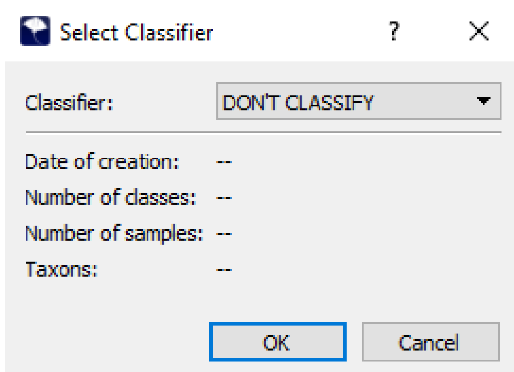


Figure 9: Selecting a classifier

While Stingray is working, the right panel will show the user the objects being detected (based on the settings from Subsection 5.2) for each plane analyzed, for each hologram. Stingray will display the number of holograms and time remaining on the bottom left panel. 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. The classifier being used in analysis and progress bar (for one pair of holograms) are shown on the bottom right panel.

6.1.1 Real- time analysis

To start analysis, click the **Start Analysis** button (or **Analyze -> Start Analysis**).

To stop analysis, the user will have to click the **Stop Analysis** button (or **Analyze -> Stop Analysis**).

6.1.2 Offline analysis

In “Offline mode”, as soon as the user selects **Analyze offline holograms**, the analysis will start right away.

In “Offline mode”, Stingray will end automatically, or the user can select **Stop Analysis** to stop analysis partway through.

6.2 Viewing Find Results

Once completed, the results of Stingray’s analysis process are presented below in Figure 10. Newly detected objects will be displayed at the top of the Results Display.

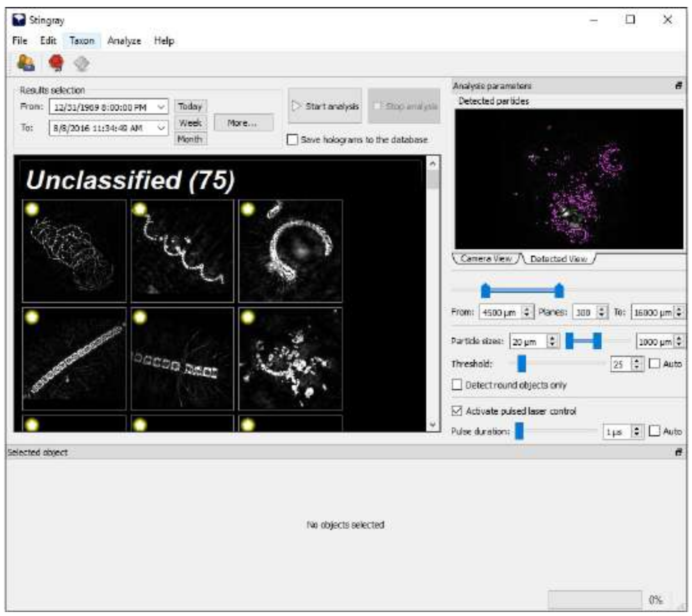


Figure 10: Display of Stingray results

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.

6.2.1 Viewing Individual Object Properties

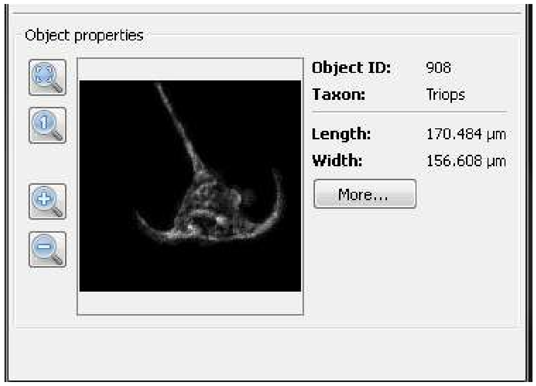


Figure 11: Selected object view and properties

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 (shown in Figure 12), and parameters for the classifiers.

Figure 12: 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`).

7 Create, Assign and Edit Taxon

At this point, Stingray will have the objects classified, as shown in Figure 13, either all in "Unclassified" or in specific taxonomic groups. As mentioned before, in practice, when first used, there will be no taxonomic groups.

Figure 13: Results from the "Find" Step

7.1 Create and Assign Taxon

Once there are objects added to the database, the user will need Create and Assign Taxon based on the objects in the database. In practice, these taxon may be very specific, down to the species level, or may be broader, such as taxon called "circle" or "rod". In all cases, the user should create a noise/junk/garbage taxon as well, for out-of-focus particles and/or of uninteresting particles.

1. Find an object in the database of a known species or genus. Click on the object (or multiple objects using Ctrl and clicking). A blue box should appear around the object(s).
2. Go to `Taxon -> Assign Taxon` (or Ctrl + T).
3. The "Taxon" drop down menu will contain all current taxa (again, in practice this will only contain "Unclassified" at first). To add a new Taxon, click the "+" to the right of the Taxon drop down menu.
   (a) The "-" will delete a Taxon, but will not delete an object
   (b) The the pencil tool will allow the user to edit a Taxon

4. There are 2 options to assign the Taxon. The **Assign taxon '[taxa]' to [#] object(s)** button will simply assign a taxon to the object. The **Assign taxon '[taxon name]' to [#] object(s) and add to samples** button will classify the object and save the object as a sample (Figure 14). Samples will be used later to train the classifier.

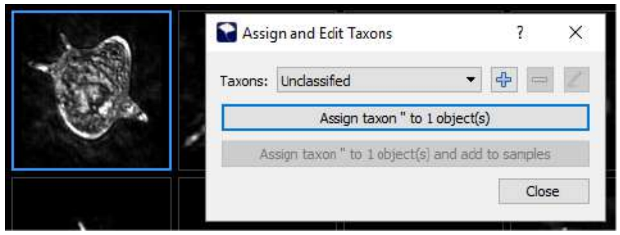


Figure 14: Creating and assigning Taxon to objects in the database

Note that the user can automatically classify objects in the database using a classifier, once a classifier exists. See Section 10.

8 Save Objects to Sample

Note that this section can be skipped if the user used the **Assign taxon '[taxon name]' to [#] object(s) and add to samples** button in the previous step.

Now the user will need to select good objects from the database that Stingray has found, and turn them into samples to be used to train the classifier.

1. Simply click on an object to add as a sample or select multiple objects using Ctrl + click.
2. Select **Taxon -> Save selected samples** or **Taxon -> Save all samples** (if the user wishes to use all objects as samples).

Figure 15 shows the results of saving an object.

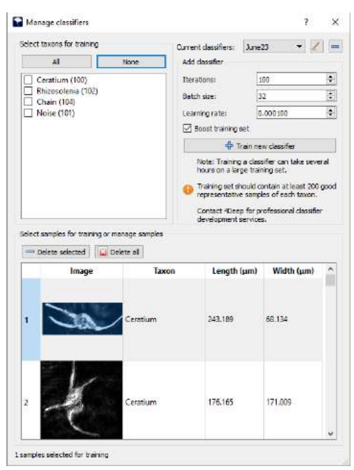
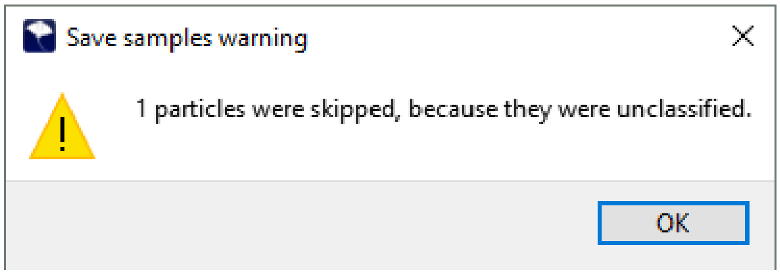


Figure 15: Samples added

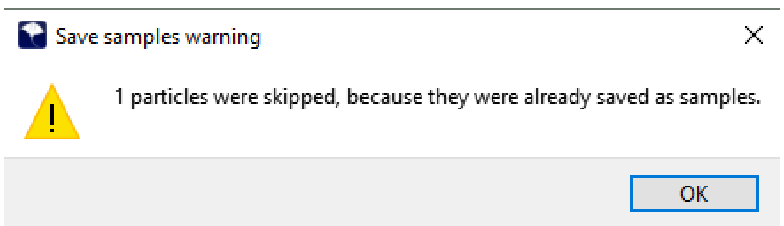
Note that the samples for classifier training are a different dataset than that of the objects in the database. Thus, once an object is saved as a sample, changing the taxon or deleting the object from the database (in the main window) will NOT change the sample. For example, if the user deletes a particle that is a sample, the sample still exists. In order to completely delete the particle from Stingray, the user will have to delete both the sample and the object.

It is recommended there are a minimum 200 samples per taxon.

Note that if the user attempts to save an object as a sample, before assigning the object to a taxon, a warning will appear:



Note that if the user attempts to save an object as a sample, that has already been saved as a sample, a warning will pop-up:



9 Train Classifiers

Initially, there will be no classifiers, so the user will have to train a classifier. In practice, users will generally use either one master classifier for all taxon (5-10) or use multiple classifiers, depending on the location or samples. For example, two classifiers may be used to classify objects into taxon for shallow water objects and deep water particles, or for lake particles and oceanic particles. In practice, a classifier should be used for every 5-10 taxon, but will greatly depend on the samples, the quality of the samples, and the number of samples available.

At this point:

- Stingray has found objects from holograms
- The user has created and assigned taxon to objects
- The user has added objects as samples to train the classifier

The next step is to choose which samples will be used to train a classifier.

9.1 Select samples to train classifiers

As mentioned above, initially, there will be no classifiers. Typically, to train a classifier, there needs to be 200 good samples per taxon, and a minimum of 50 to train to begin. Typically, (but dependent on the taxon of interest) many holograms (thus many samples with the microscope) are needed to generate 200 good samples per taxon.

Figure 16: Selecting good particles from the results of “find”. Note that the red boxes do not appear in Stingray.

9.2 Training Classifiers

Users will 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`**, shown in Figure 17) is used to train classification systems to identify selected objects based on morphology parameters.
  - Once the dialog is open, objects selected as samples in the Results Display will be listed along with parameters of the object.
  - The user can delete samples selected in the table with Ctrl + click or Shift + click and click **`Delete selected`** or the **`Delete all`** button, which will delete all.

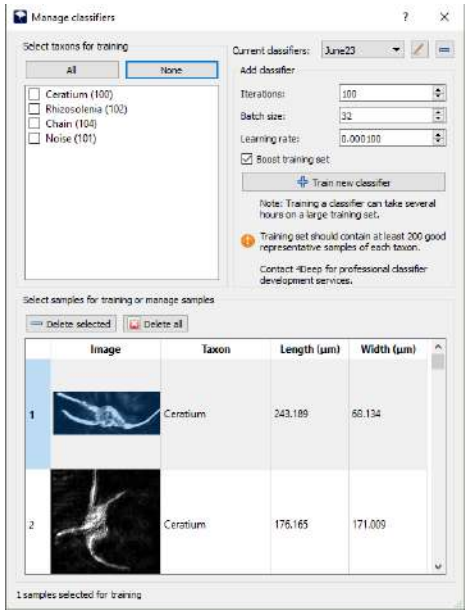
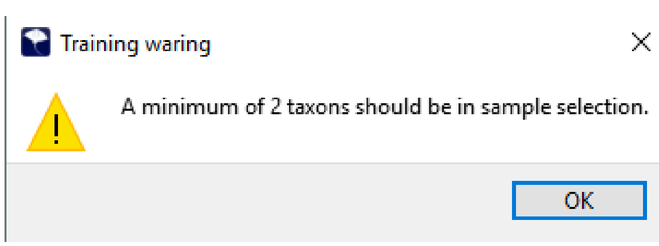
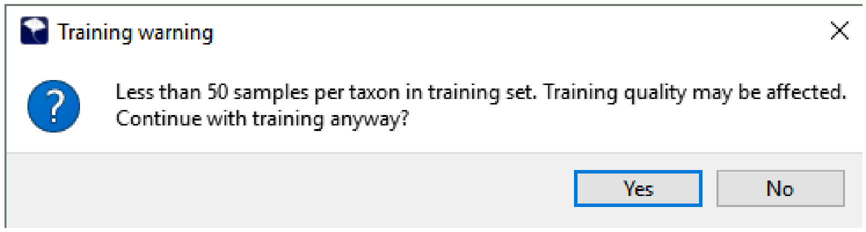


Figure 17: Manage classifiers dialog

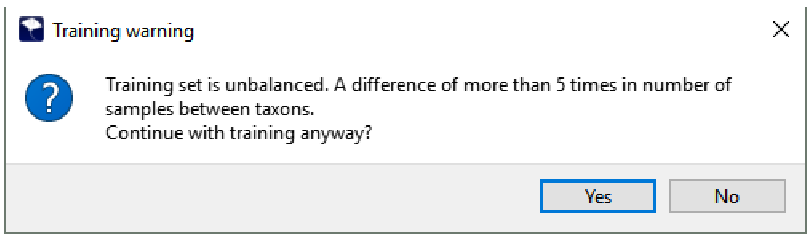
- Input options for classifying
  - **Iterations:** number of training passes used to train a network. Each iteration presents the entire set of selected samples to network. 200 is the default.
  - **Batch size:** : the number of samples that are presented to network in a single portion. 32 is the default. Use smaller number if you run out of memory during training. Use larger size if network does not converge or accuracy changes randomly from one iteration to the next.
  - **Learning rate:** determine how quickly the weights in the network are updated. Default is 0.001. If the accuracy of training does not change in 20 or more iterations, increase the Learning rate by a factor of 10. If accuracy changes randomly or network does not converge, decrease the Learning rate by a factor of 10.
  - **Boost training set:** artificially expand the training set by a factor of 3 by flipping sample images horizontally, vertically and in both directions.
- Choose the samples to be used in the training, ensuring that at least two different taxa are selected. Once all selections have been made, the `Train` button will set the classifiers to be trained using the defined parameters.
  - Note that at least 2 taxa are required to train classifiers. A warning dialog will pop up if this is the case. See Section 7 for instructions on how to create a taxon.



  - Note that multiple good-quality images (minimum of 10, but at least 200 to get reasonable results) of the same species are necessary to add to the samples for good classification training. See Section 9.1 for instructions on how to add samples.



– Note that there needs to be approximately the same number of samples in each taxon to “balance” the classifier. See Section 9.1 for instructions on how to add samples.



- A training dialog will appear (shown in Figure 18)

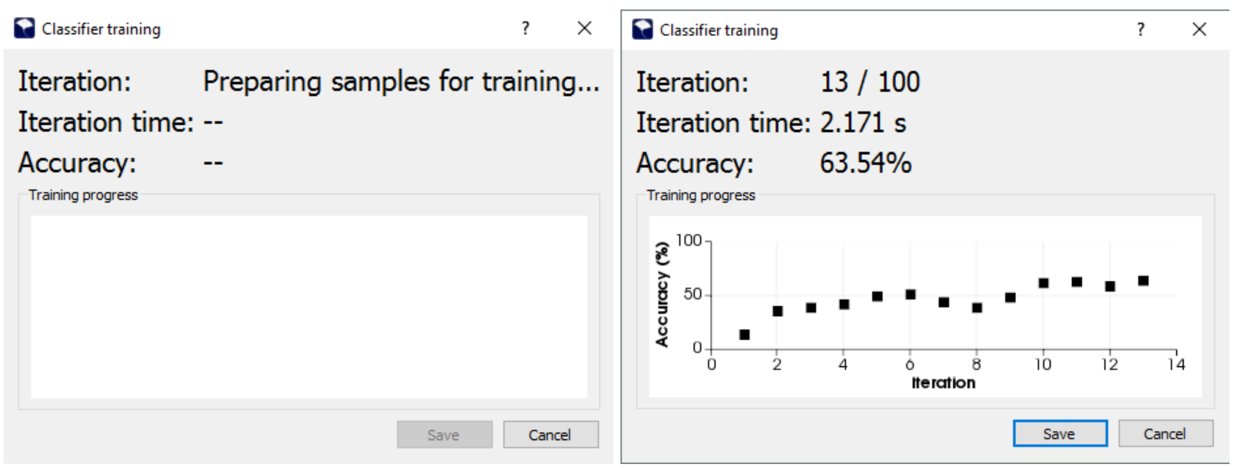
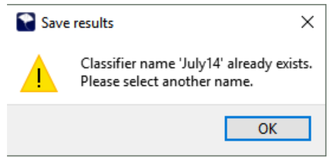


Figure 18: Result of Train. Left panel is Stingray preparing to train the classifier. Right panel shows the iterations during training.

- During training (right panel of Figure 18), the classifier should converge to 90% or more. If the classifier converges to 100% quickly, for example, in less than half of the iterations, decrease the Learning factor by 10 and try again. The user can also cancel the training by clicking **`Cancel`** (the classifier will not be saved) or accept the current classifier before all iterations have completed by clicking **`Save`**. This Save option is useful if the accuracy converges to 100% before all iterations have been completed.
- The user will need to name the classifier. Suggestions for a name include: the date, the species used, etc. The user should make note of the number of taxon, number of samples, and date the classifier was created for future use and comparison purposes.

  If there is more than one classifier, the user will need to give the classifiers different names. If the user tries to re-name a classifier, s/he will be prompted to to choose another name:



- Once trained, the classifier is added to the list on the upper right, under **Current classifiers**
- If the classifier is unsatisfactory (see Subsection 9.3) 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 (see Section 10)

Note that the classifier uses deep neural network for classification, based solely on the sample image, not on morphological parameters.

9.3 Verify the classifier is efficient

At this point, there are objects in the database, samples that have trained the classifier, and a classifier exists. Once classifiers are trained, the user will need to run samples with the classifier to ensure that it is successful. For example, if there are two taxon, "good objects" and "bad objects", select a couple of good particles and a couple of bad particles in the "Unclassified" taxon, and classify using **Taxon -> Classify selected particles**, selecting the classifier that needs to be tested. The user can determine if Stingray classifier these particles correctly by simply navigating to those two taxa and looking to see if the particles are classified well.

Important *If the classifier does not perform well, training must be done again*. The parameters can be changed: increase the number of iterations (default is 200), decrease the Learning rate by a factor of 10 (default is 0.001) and/or boosting the training set. 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. If a classifier does not work well, the user can delete it by going to **Taxon -> Manage classifier**. In the drop-down menu, select the classifier to be deleted and click the "-".

Repeat the tests with the newly trained classifiers, and if results from the classification are satisfactory, the classifiers are ready. With functioning trained classifiers, Stingray can now operate as an automated morphology detection and classification program.

If a particle is classified incorrectly, simply click on the particle and **Taxon -> Assign taxon**, reassign the object to "Unclassified". Note the user must re-classify an object as "Unclassified" in order to give it a different taxon.

Note that once an object is assigned to a taxon automatically, it is not automatically saved as a sample, and will have to be saved as a sample

10 Run Classifier on Objects / Holograms

In practice, the classifiers will be used in two ways. First, the user can select object(s) already in the database to classify. Secondly, the user can analyze a new set of holograms, running the classifier on objects from the holograms, simultaneously, as Stingray find objects.

10.1 Run classifier on Objects in Database

The following are the steps to classify objects already in the database:

- To select or change the classifier, use `Taxon -> Select Classifier` or select "Ctrl + L".
- Select objects from the database to classify, by clicking or Ctrl + clicking to more than one.
- Go to `Taxon -> Classify selected particles`. A dialog will pop up, allowing the user to decide whether to accept the classification.
  - The user can "Select all" or "Select none" to accept the classification of all or none of the taxa assignments
  - The user can change the "Select objects with score >=", as it controls the minimum score or certainty the classifier has that the object falls in that taxon. If the user changes the minimum score, Stingray will save the value for future use. Stingray will only assign taxon to objects that meet the minimum score.

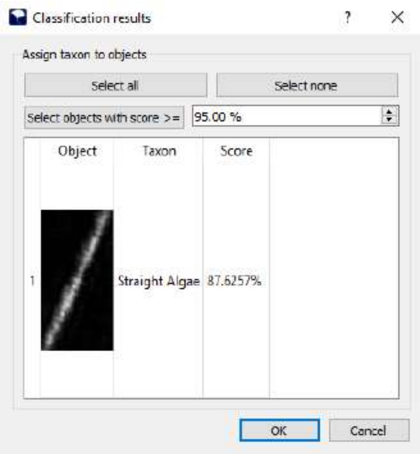


Figure 19: Dialog for accepting the classification.

- If accepted, the objects will appear in the taxon categories according to the classifier
- If a particle is classified incorrectly, simply click on the particle and `Taxon -> Assign taxon`, reassign the object to "Unclassified". Note the user must re-classify an object as "Unclassified" in order to give it a different taxon.
- Note that once an object is assigned to a taxon automatically, it is not automatically saved as a sample, and will have to be saved as a sample

10.2 Run classifier when finding objects in holograms

To run a classifier on objects as Stingray finds objects, the user will need to "Analyze offline holograms". When the holograms are selected, a dialog will pop-up in Stingray, prompting the user to select a classifier. If the user wants to use a classifier, simply select the classifier from the drop-down menu.

If the chooses not to use a classifier, simply select "Don't Classify".

By default, a taxon will be assigned only if classification score > 90%, otherwise it will remain as unclassified.

If a particle is classified incorrectly, simply click on the particle and **Taxon -> Assign taxon**, reassign the object to “Unclassified”. Note the user must re-classify an object as “Unclassified” in order to give it a different taxon.

Note that once an object is assigned to a taxon automatically, it is not automatically saved as a sample, and will have to be saved as a sample

11 Using Stingray Regularly

Eventually, once a classifier (or classifiers) is trained properly, the user will be able to simply analyze holograms and run a classifier on the holograms, without needing to collect more data or add more samples to train the classifier.

12 Exporting

The user can select objects to save from the database by clicking to select objects (Ctrl + click for more than one) or Ctrl + A, to select all. Then go to **File -> Export images** (or use Ctrl + S).

The user can also select objects to save in an Excel spreadsheet by clicking to select objects (Ctrl + click for more than one) or Ctrl + A, to select all. Then go to **File -> Export report** (or use Ctrl + R). For each object, the report contains all of the information detailed in “Object Properties” from Stingray.

13 Summary of Steps

1. Analyze holograms collected in some body of water offline or online. For offline analysis, see the Octopus and microscope (Submersible, Desktop or Cuvette) user guides. Once enough data is collected and stored in the database, proceed to step 2. Ideally you need several thousand good objects at this stage.
2. Assign taxa to known objects (Section 7)
3. Assign noise and out-of-focus particles to a “noise” taxon. There may be more than 1 of these (ie one “Noise” and another “Out-of-focus”) (Section 7)
4. Of the objects assigned into taxa, save good representative objects as samples, including the “noise” taxon as well (Section 8)
5. Once you have assigned enough samples (200 or more for each taxon), go to Manage classifiers
6. In Manage classifiers select taxa you want to train to detect, click Train new classifier (Section 9)
7. Wait for classifier to be trained, accuracy should be > 90%
8. Test classifier by selecting objects in the database and running a classifier (Subsection 9.3). Check the results, note what objects are being mislabeled. If results are not satisfactory it can be because of:
   - poor quality or not enough samples
   - errors in selecting classifier training parameters
   - some overlaps in image appearances between taxa
9. If classifier performs well, it can now be used in automated online or offline analysis

14 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
Warning appears stating there needs to be at least 2 taxa to train a classifier	Create at least 2 taxa, and add samples to each.
Warning appears stating there are not enough samples in a taxon	Add at least 50 samples to each taxon for classification
Warning appears that the training set is unbalanced	Add the same (or close to the same) number of samples to each taxon. For example, 50 of taxon A and 60 of taxon B should be fine, but 50 of taxon A and 500 of taxon B would lead to the classifier favouring taxon B in classification
Warning appears alerting the user the particle was skipped when trying to add an object as a sample	Assign a taxon to an object before adding it as a sample
Classifier incorrectly classifying objects	Ensure there are at least 3 taxon - 2 species of interest and a “noise/junk/garbage” taxon. Ensure there are at least 200 samples in each taxon. Ensure the training accuracy of a classifier is at least 90%. Boost the training set.

Table 1: Common troubleshooting problems and solutions

15 Appendix

15.1 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).

`START_ANALYSIS\n`

Starts the analysis acquiring holograms from the connected camera.

`STOP_ANALYSIS\n`

Stops the analysis.

ANALYZE_HOLOGRAMS Val\n

Starts the analysis on the list of hologram files. Val is a list of hologram names, delimitied by *. Each name can be either an absolute path to hologram file, or a name relative to the directory in which offline hologram files were loaded from. A minimum of 2 file names are required for analysis.

EXPORT_IMAGES Val\n

Exports all currently visible in the image grid objects as image files. Val is a name of the directory in which images will be exported. Images are exported in PNG format.

QUERY_DATABASE Val\n

Queries the database and displays the list of objects on the grid according to the query. Val is a list of query parameters, delimited by space " ". First parameter is "date from", in the format of "yyyy-MM-ddThh:mm:ss". Second parameter is "date to", in the format of "yyyy-MM-ddThh:mm:ss". Third parameter is a list of taxon names, delimited by |.

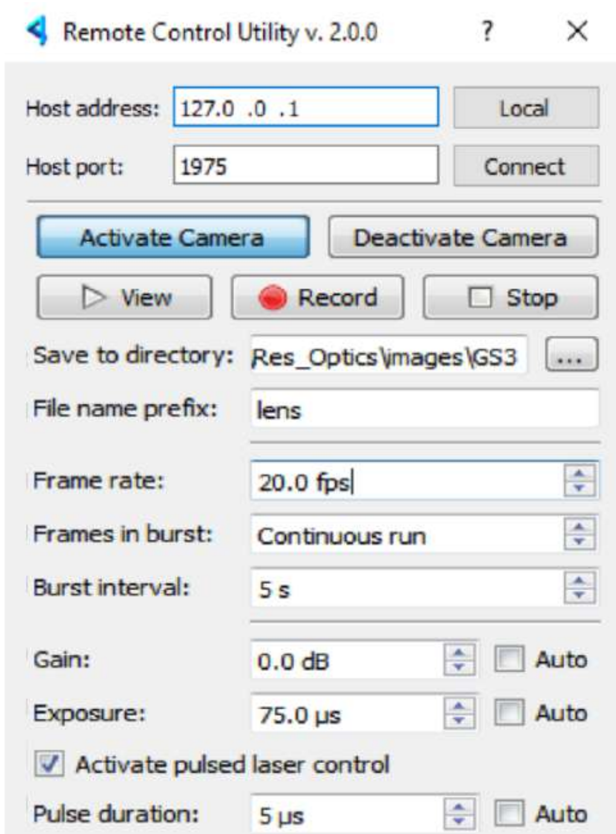


Figure 20: Camera Remote Control Utility

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.

15.2 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.

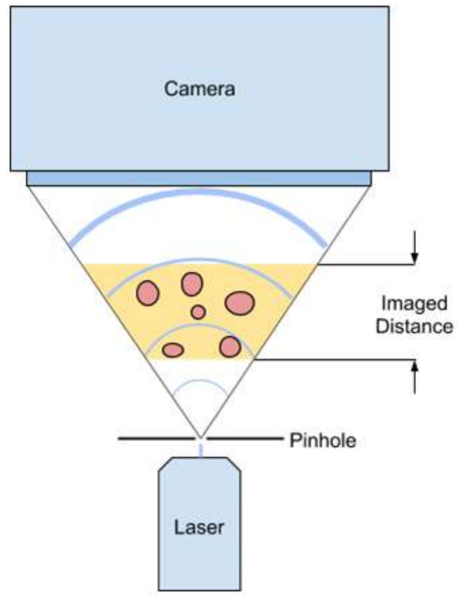


Figure 21: The basic principle of digital in-line holographic imaging

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