
Holodetect User Manual

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Made:	Holodetect Instruments Ltd. Barbara Bicsák László Orzó László Nemes
Reviewed:	Ákos Zarándy

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Document history

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Barbara Bicsák	2024.04.24	1.1	Step-by-step measurement demonstration	IT manager

Introduction

Detecting microparticles, microorganisms, counting various cell species in liquids to ensure quality is paramount across multiple industries, including microalgae culturing, food production, water utilities, and beyond. A wide range of technologies and methodologies are available to measure critical parameters of these processes. However, many of these solutions can be labor-intensive, costly, and challenging to integrate into existing industrial workflows.

Holodetect devices have been developed to alleviate the need for using manual methods for cytometry, offering a significant advantage in scaling up operations in microalgae farming, food processing operations or ensuring water safety for water utilities. Automation in quality control is crucial for cost efficiency and maintaining consistent quality. Our devices are customizable to meet the specific needs of various species, microparticles, and can flexibly integrate with a wide range of industrial equipment. The developed instruments feature measurement cuvettes with depths ranging from 100 to 10,000 microns to accommodate different magnification needs.



Technology

Leveraging the power of digital holographic microscopy (DHM), Holodetect captures the entire depth of a sample volume in a single image, eliminating the need for refocusing. This technology allows for reconstructing all sample objects from the recorded hologram image with minimal sample preparation. It also supports flow-through operations for analyzing large volumes of samples.

Advanced artificial intelligence powers the analysis of reconstructed images. These AI models are highly adaptable and can be trained to accurately identify various species and objects within the sample. Additionally, our holographic microscope can be integrated with fluorescent microscopy to enhance classification precision and conduct a variety of

fluorescence-based measurements. This synergy between technologies ensures comprehensive sample analysis with unparalleled accuracy.

Sample Preparation, Limitations, Constraints

DHM implements volumetric in vivo imaging of the samples, thus it does not require the “flat mounting” of the sample using centrifugation or sedimentation. As DHM requires the majority of the reference beam to pass through the sample unscattered, it also poses an upper limit on the density of the sample. For high resolution devices using 200 micron deep cuvettes (Holodetect HiRes, HiRes + Fluor), it is about 400 objects/ μL (400,000 object/ml), which is actually the typical algae/object density of a natural surface water sample. For high volume devices (Holodetect HiVol) using 10,000 micron deep cuvettes, it is about 140 objects/ml.

Samples of higher concentrations must be diluted to below these levels prior to the measurement. The optimal concentration in terms of measurement speed should be around 100-300 objects/ μL for the HiRes devices and 80-120 objects/ml for the HiVol devices.

The fluorescent channel in Holodetect HiRes + Fluor uses a lower resolution microscope than the DHM due to its lower focal depth to capture the same approximate volume of the sample. For the two-channel imaging and fluorescent-aided classification to work properly, the field-of-views of the two microscopes need to be precisely aligned. Hardware alignment is done at the premises of Holodetect Instruments, or at the customer’s premises by Holodetect personnel.

The further alignment may be needed in certain time intervals is supported by the DHM’s software. Please find a detailed description in the *Calibration* session.

The built-in fluorescent microscope enables the label free detection of autofluorescent molecules in cells. By default, the microscope uses a laser for excitation of Chlorophyll A molecules with a wavelength of 480 nm. This can be customized upon request to other excitation wavelengths, e.g for specific dyes used for labeling the sample.

Due to the fluorescent microscope’s lower resolution and single excitation wavelength, the DHM application can only calculate an approximation of the Chlorophyll content of the cells and may need to be calibrated using other measurement methods.

Comparison of Holodetect Devices

Technical data of currently available products are given in the following table:

	Holodetect LVol	Holodetect HiRes	Holodetect HiRes+Fluor
Dimensions (W x L x H)	30 x 40 x 22 cm	30 x 48 x 35 cm	30 x 48 x 35 cm
Weight	6 kg	7.5 kg	8 kg
Detectable object size range	15 - 300 µm	3 - 75 µm	3-75 µm
Holographic Resolution	2 µm	600 nm	600 nm
Measured volume	10-50 ml/min	3,6-18 µl/min	3,6-18 µl/min
Flow-through cuvette depth	10 mm	200 µm	200 µm
Particle size and shape statistics	✓	✓	✓
AI based classification and counting	✓	✓	✓
AI training module (optional)	✓	✓	✓
Built-in annotation tool	✓	✓	✓
Automatic reports	✓	✓	✓
Remote access	✓	✓	✓
Fluorescent detection accuracy	-	-	4 µm
Excitation wavelength	-	-	445 - 480 nm
Emission wavelength	-	-	500 nm <

DHM only functions on Holodetect HiRes and HiRes+Fluor are identical.

Few-Shot Learning Module

The classification function in the DHM application uses deep neural network models. The Holodetect device you ordered may be shipped with a few models pre-trained for specific species/classes (e.g. Scenedesmus, certain fungi species, etc.). For other species and custom classes defined by you, model training is available as a service from Holodetect Instruments. To train these relatively large models to achieve low classification error, we leverage our extensive computational resources. Attaining the required accuracy, however, also requires a large training database, typically supplied by you. This can be a tedious task and may require lengthy measurements, as you must provide a balanced number of examples for each class ranging from hundreds to thousands of labeled (manually classified/validated) objects, using the built-in annotation tool in the DHM application.

Few-Shot Learning is an optional module of the DHM application to alleviate the task of preparing training databases, and to enable the training of models on the DHM's built-in computer, at a reasonable trade-off of accuracy. There are three levels of model training available, with increasing computational complexity, and required training database size. The level 1 "Small Head" model training requires as few as 10 examples/class. Using the model pre-trained by level 1 learning, you can pre-classify a larger number of objects reducing the need for annotation for level 2 or 3 learning. Level 2 (Large Head) requires 100-200

examples/class. Similar to level 1, using the level 2 trained model, a large training database can be prepared for level 3 learning (Full Model) requiring at least or more than 100 examples/class. Levels 2 and 3 separate a validation set to avoid overfitting to the training data and stop the learning process automatically, while stage 1 is trained on all samples for a fixed 40 epochs.

A detailed description of how to use the Few-Shot Learning Module can be found in the *Using the Few*Shot Learning Module* section.

Sample input

The sample can be loaded to the Holodetect device using a standard falcon tube. Put the sample into a falcon tube and screw it in the tube holder. Next, rotate the tube holder upside down and you are ready to start the measurement. This built-in sampling device ensures the homogeneity of the sample, preventing its sedimentation.



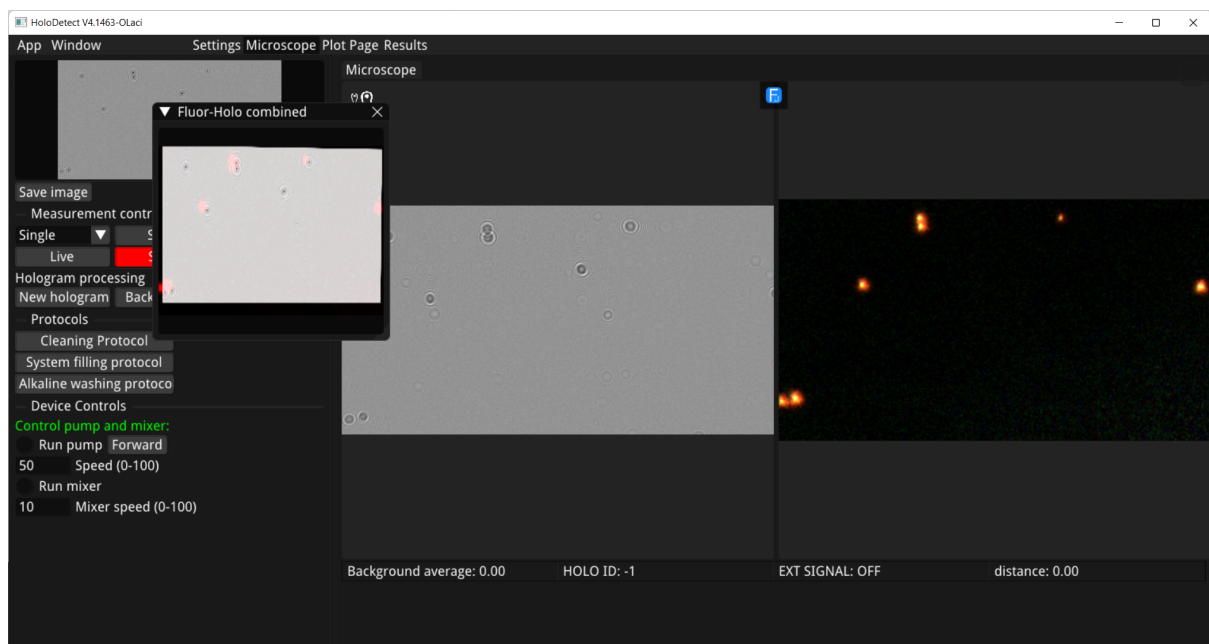
After you have finished the actual measurement the system must be cleaned by transfusing, rinsing it with clean water.

The measured sample and cleaning, rinsing fluids are collected in a waste vessel. This waste should be periodically discarded by the users.

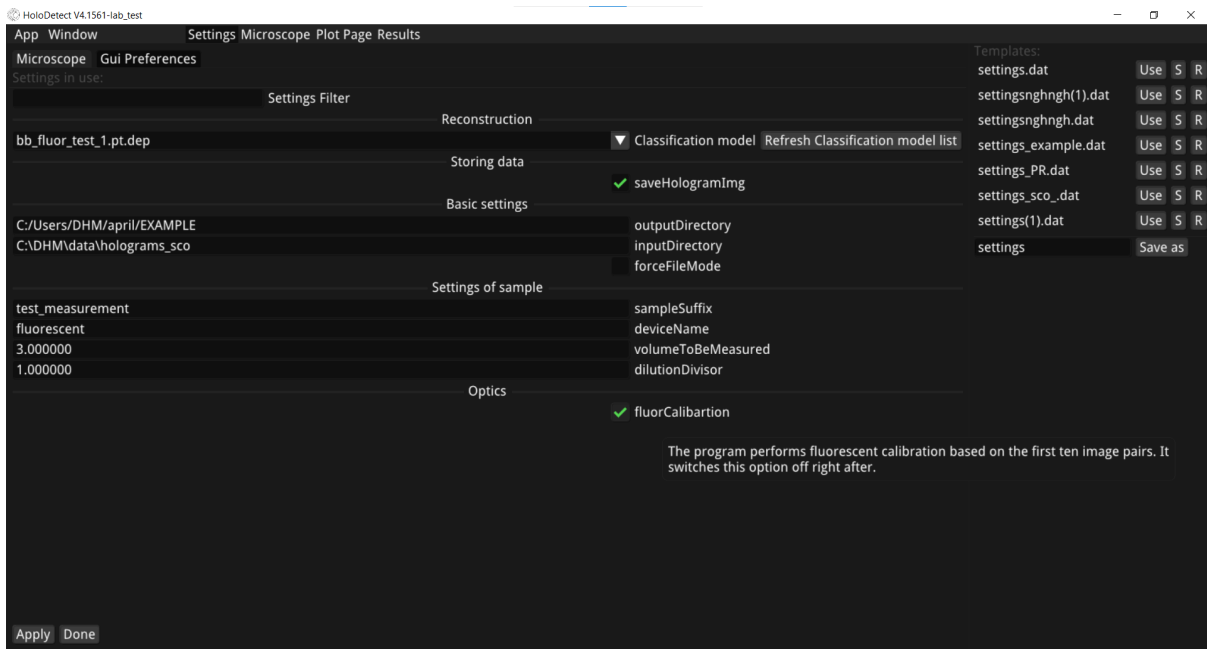
You also can use your own sample holder by connecting it to the inlet/outlet tubing of the Holodetect device. In this case the sedimentation of the sample must be prevented by some other methods.

Calibration session

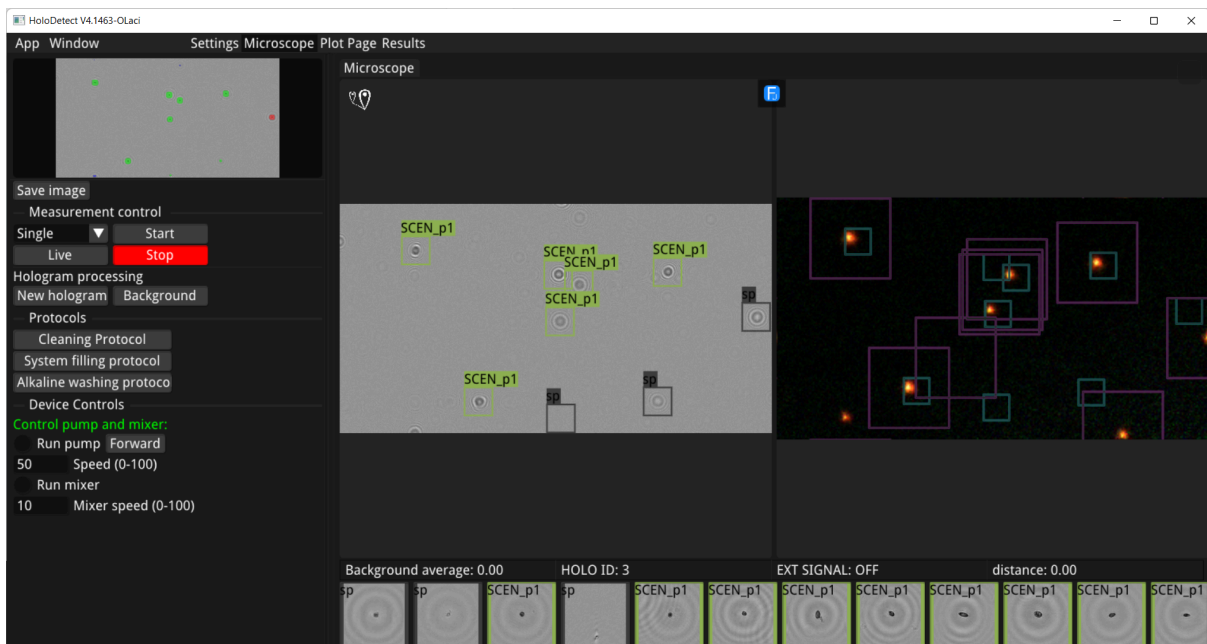
As there are two different cameras, their actual view of the sample has to be matched. This is done by calibration. The magnification difference and the rotation of the two views are set and optimized during the device construction using calibration tools that can be achieved in the advanced mode only. The light distribution of the laser that produces the fluorescent excitation is also set and compensated during the device construction process. However, there is some offset between the two cameras that changes with the device temperature and the actual position of the flowthrough measuring cell.



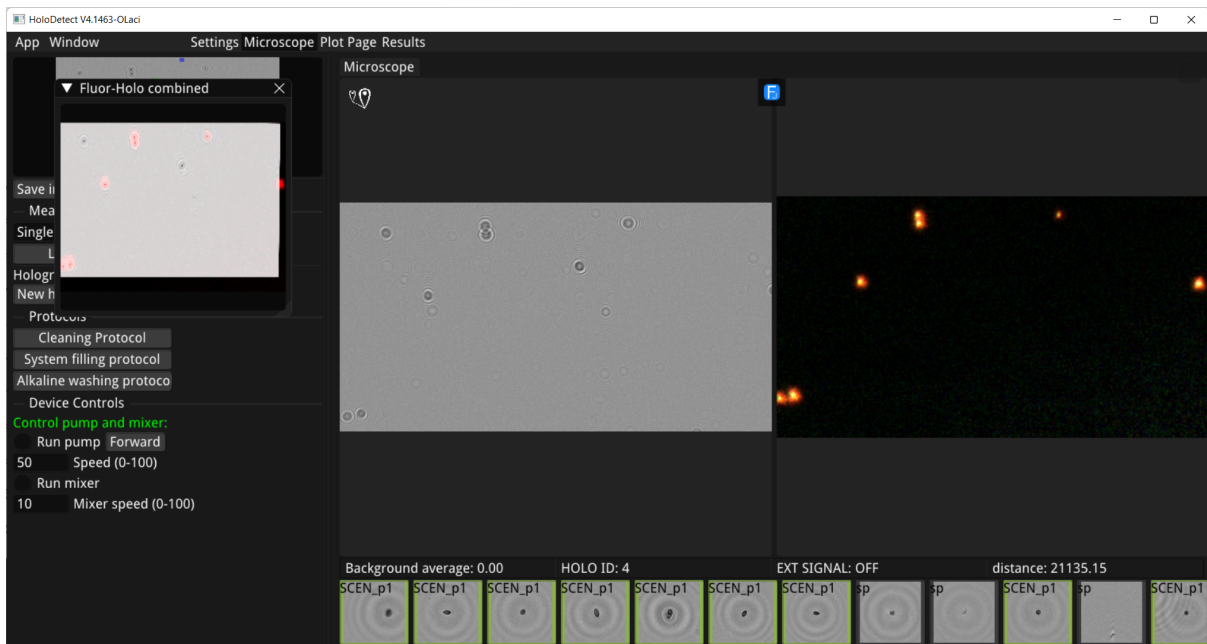
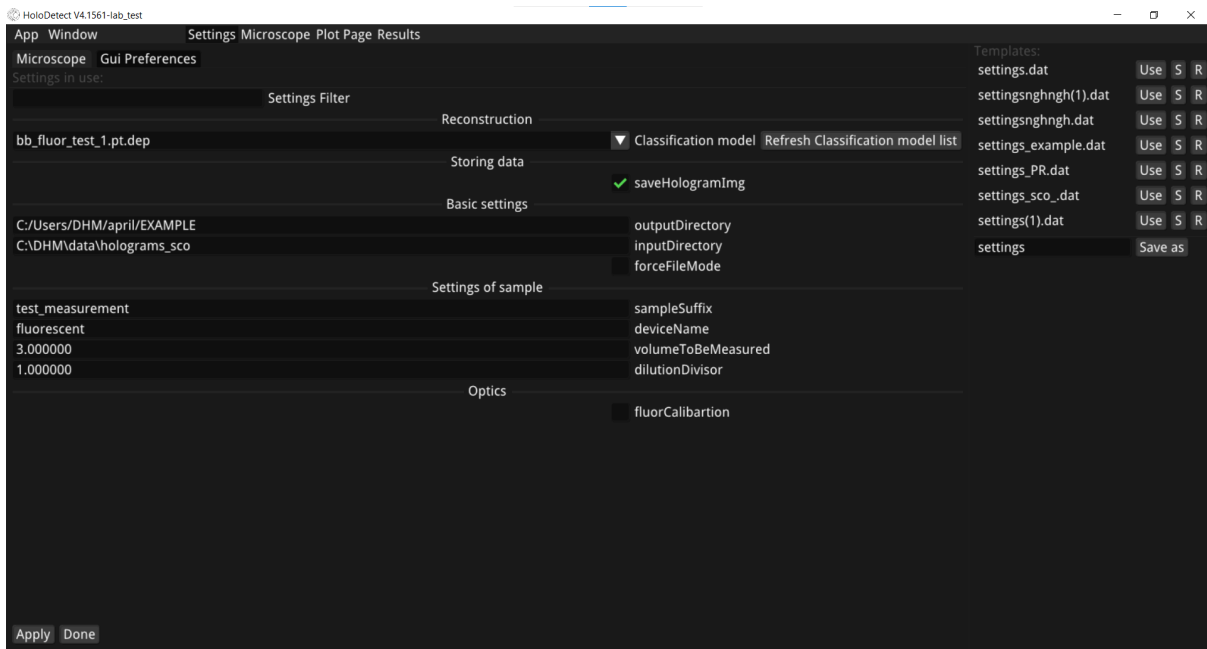
This offset has to be corrected using the built-in auto calibration routine. This requires the application of a sample that has objects with fluorescence (a sample containing alive algae is suitable). When the device is switched on or you want to calibrate the offset, please enable the auto calibration in the basic setting panel (see later).



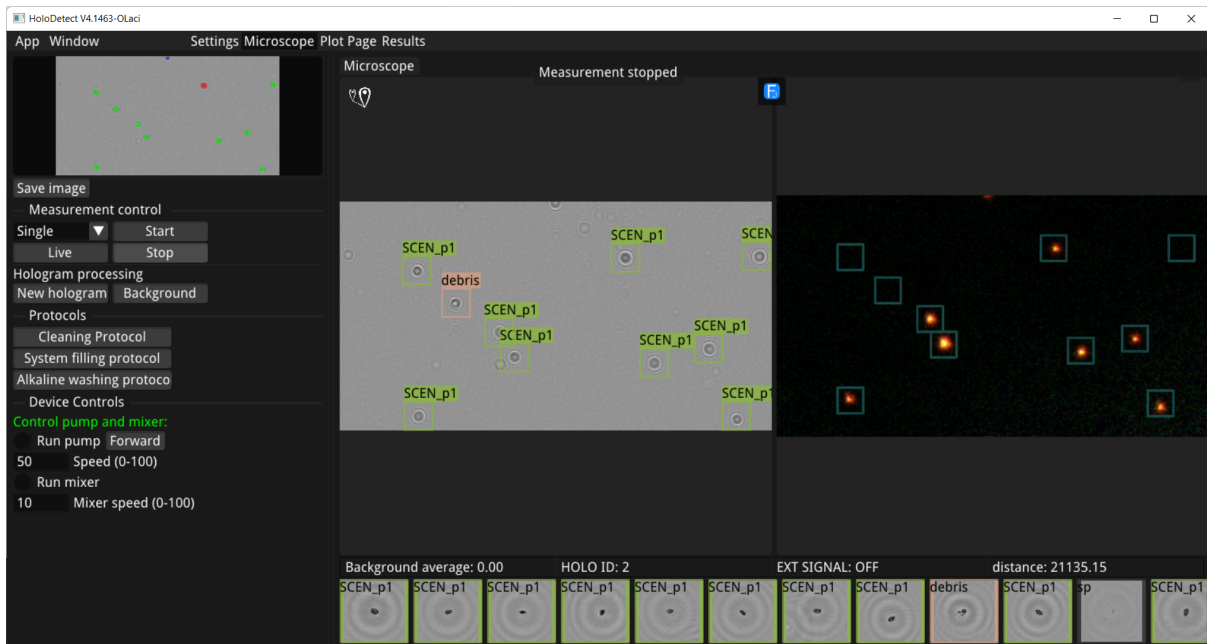
Then start a measurement. At the end of the measurement the offset will be corrected.



To validate the calibration, take a new hologram (see later). When a new hologram has been taken, in an appearing new window you can check if there is correct overlap between the reconstructed holographic objects and their measured fluorescence signal. If the overlap is correct the calibration process can be finished.



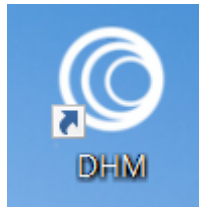
Please, save the actual settings to ensure the calibration for consecutive measurements. If the calibration is apparently not satisfactory, please run the calibration process again (with the previously saved settings - or - if the mismatch is small - with the current settings).



The Software of the DHM

Starting the DHM application

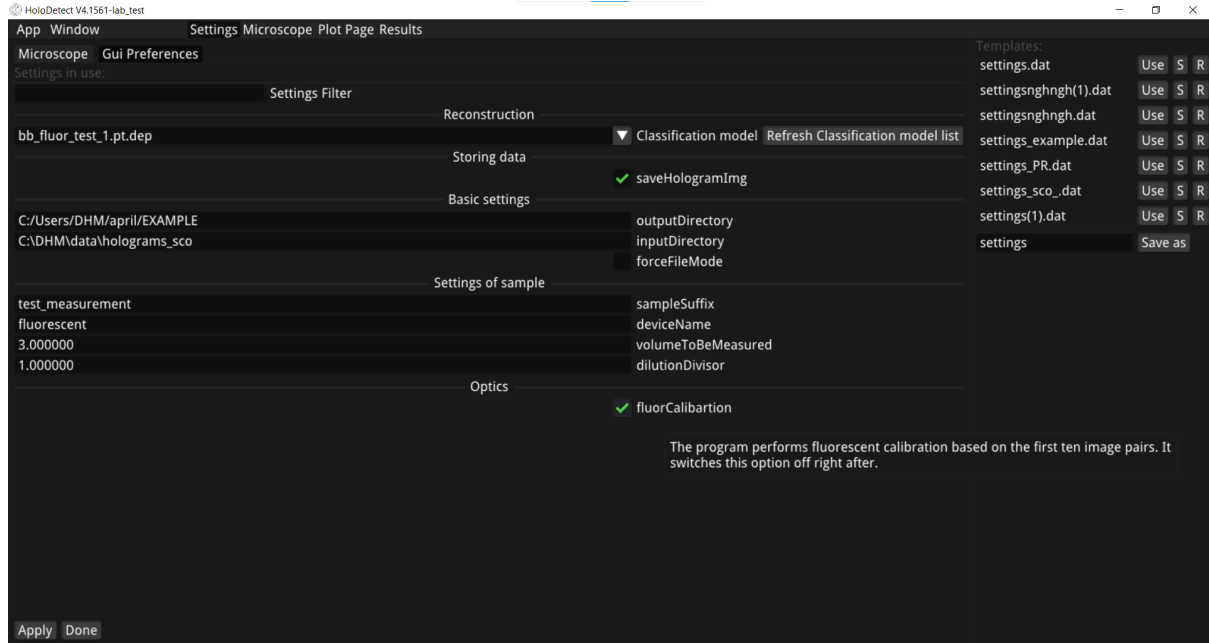
Click on the icon to start the Holodetect's Digital Holographic Microscope (DHM) application.



Settings page

- When the program starts, the *Settings* page is displayed first. Here the basic parameters of the measurement can be set: the *short name of the measurement*, the *volume to be measured (in μL)*, the *output folder* where the measurement result will be stored, and the *model that is to be used for classification*.

- If the *Done* button is pressed, the parameters set so far are enabled and the program automatically switches to the *Microscope Page*.



Settings listbox

We can choose from a list of earlier defined, saved and renamed Settings. Otherwise, we shall use the previously used one (Last settings from app). In case of using a HiRes+Fluor device, but our measurement does not require fluorescent information (the sample is not fluorescent), we can select here an alternative HiRes only Setting file and our device will work in Hires operation mode in the following. This way we can change the operation of the device.

Measurement panel

- **Classification model:** a list from which the classification neural network model we want to use can be selected.
- **saveHologramImg:** the recorded holograms during a measurement will be saved (to the folder associated with that measurement) if this checkbox is checked. They can be used to reclassify in File Mode - with different classifier if desired. In normal

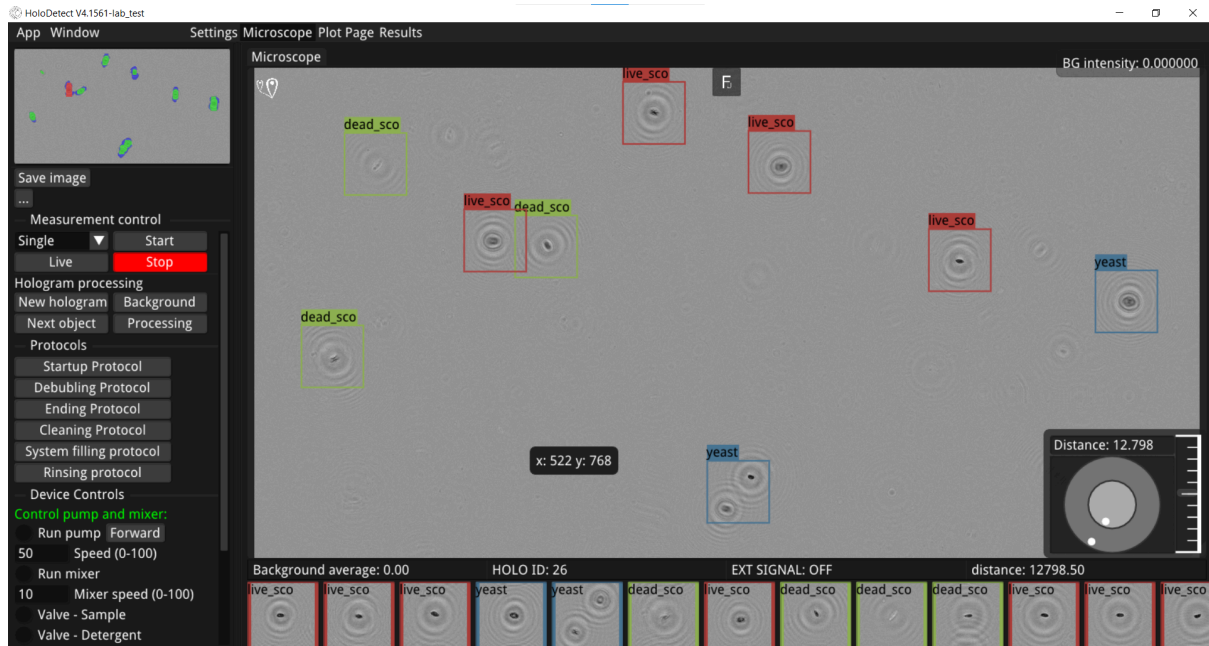
operation the holograms are not saved, only the images of the objects, because the entire holograms use significant disc space.

- **outputDirectory:** the path specified here defines the folder of the measurements. All the resulting data of the actual measurement will be stored here.ú
- **inputDirectory:** the path and the folder that contains the holograms that are to be processed in File Mode. This is used only in Forced File Mode!
- **forcedFileMode:** DHM can do the whole processing on previously measured and saved holograms by checking this checkbox. This mode is useful for demonstration or for evaluating the trained models in an iterative training process.
- **sampleSuffix:** give a short name to the measurement. This name can identify what type of measurement is done. If there are more than one measurement, the consecutive measurements get a unique 3 digit number to be identifiable.
- **deviceName:** defines the name of the device. If there are more than one in use, this name can identify which one made the actual measurement.
- **volumeToBeMeasured:** the Holodetect HiRes and HiRes+Fluor measures a sample volume of 0.12 µL per hologram, this should be taken into account when setting this value. E.g. if you set this value to 12, it means that ~100 holograms will be taken and 12 µL volume will be measured. Measurement time of a hologram is a few seconds, depending on the number of objects on it. Holodetect LVol measures a 345µL sample volume per hologram. It's important to note that only a fraction of the sample flowing through the cuvette is actually measured, due to the fact that the microscope's field of view is much smaller than the width of the cuvette.
- **dilutionDivisor:** The volume is divided by this quantity in the concentration calculations. E.g.: by 1:20 dilution its value is 20. It defaults to 1,0..
- **fluorCalibration:** When the device is switched on or you want to calibrate the offset, please enable the auto calibration in the basic setting panel (see later). The program performs fluorescent calibration based on the first ten image pairs. It switches off right after.
- On the right, different letters indicate different options:
 - **Use:** the selected Settings file will be loaded by the system.
 - **S:** the selected file will be saved.
 - **R:** rename the selected file.
 - **D:** duplicate the selected file

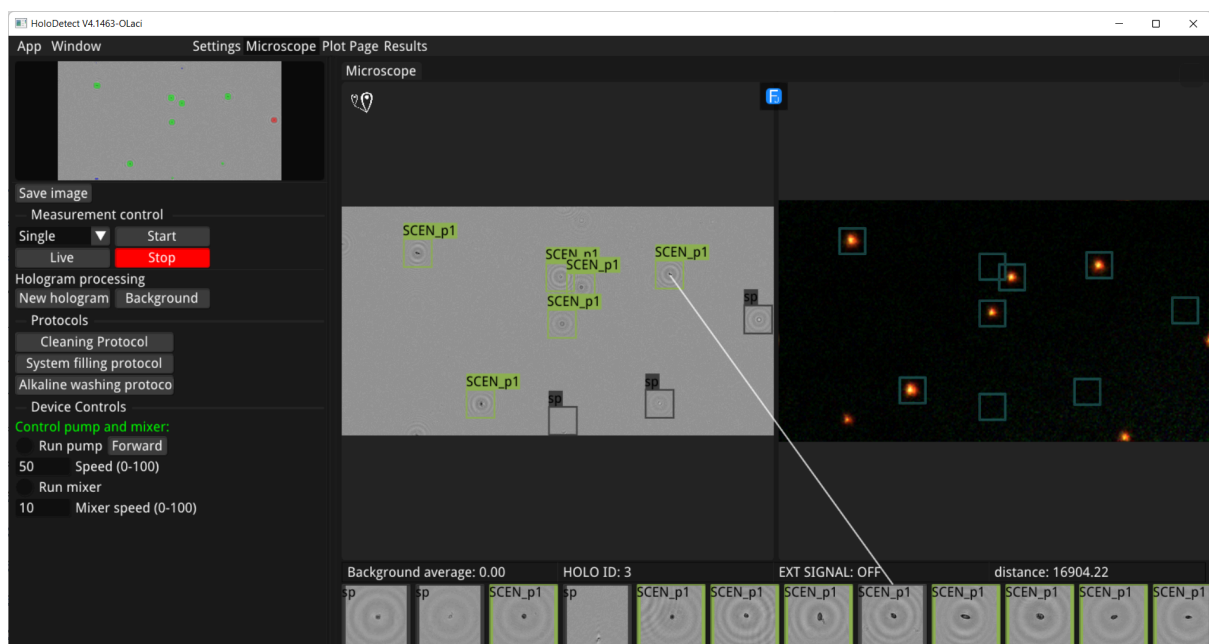
- **X**: delete the selected file.
- **Save as**: the loaded Settings file can be saved with a new name.
- **Apply button**: Enables the so far set parameters.
- **Done button**: Enables the parameters set so far and moves the focus from the Setting window to the Microscope window, where the measurement can be initiated.
If you do not press either Apply or Done button, your settings will not be used!

Microscope Page

The measured holograms are displayed in the central panel of the window, where the Holodetect label is shown at startup.



By clicking the central Fluor icon (capital F) the fluor display will also appear next to the hologram. The centers of the measured fluorescent and hologram objects should be aligned.

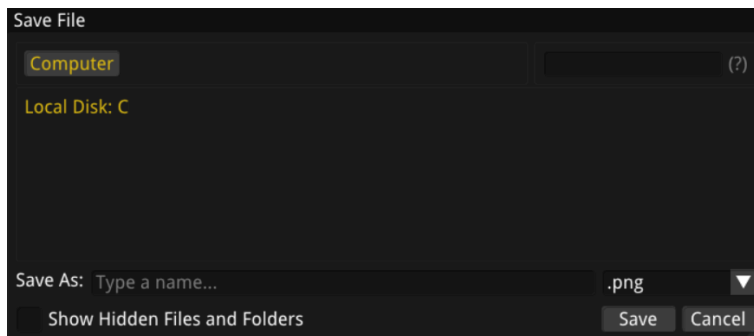


This can be achieved using the built-in auto-calibration tool (See Calibration session!).

During the measurement the objects are displayed in the bottom of the window as they are being processed. The main features of these objects (class, reconstruction distance, etc.) can be found here too. By clicking on the small icon of a reconstructed object moves the focus of the whole hologram in the reconstruction distance of this object and points their position by a line segment.

All measuring and microscope handling tasks can be controlled by buttons that are located on the left panel of the window. These buttons are as follows:

- **Save image** - saves the currently displayed image in the central panel in png format. It is usually the measured hologram, but it is possible to focus the hologram before saving using the focus knob.



Focus Knob Window

There is a Focus Knob in a standalone window, which works in a similar way that of the conventional microscopes' and makes the manual focusing of the holograms possible. It contains fine adjustment and coarse adjustment knobs, which allow you fast and accurate manual focusing. Beside these ones, there is a focusing slider, when large scale focus adjustment is required.



The actual position and parameters of this window can be chosen by pushing the right mouse button and selecting the proper element from the list.

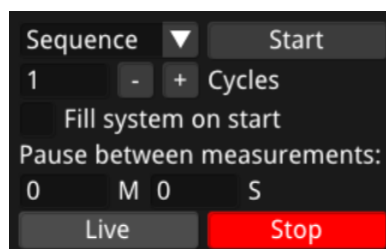
Measurement Control

- In this panel you can set the actual type of measurement:
Single, Sequence, Interval, Continuous.
- In *Sequence, Interval* and *Continuous* modes, it is possible to set a parameter, *Pause between measurements*: this determines the time interval between each measurement, given in minutes (*M*) and seconds (*S*) (this is optional, by default, there is no pause between two consecutive measurements).

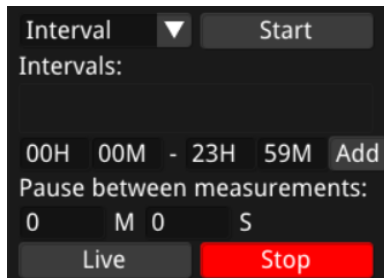
- *Single*: In this case, a single measurement will be taken.



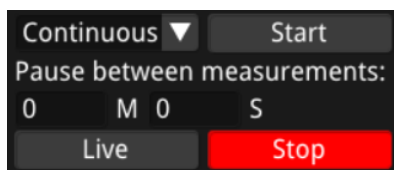
- *Sequence*: In this case, the DHM takes a series of measurements from the given sample. The *Cycles* value should be set according to the number of the consecutive measurements. Then, by selecting the *Fill system on start*, enough samples can be loaded into the system (if a sample will be measured in *Sequence* mode that will already be in the system, therefore this step is skipped).



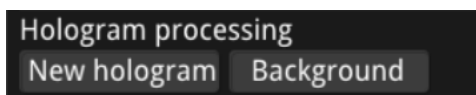
- *Interval*: It is possible to specify the different intervals at which measurements should be run (in hours and minutes). The first value of the interval indicates when the measurement starts, the second value indicates when the last measurement will start (i.e. not when the measurement will stop). More intervals can be created by clicking on the *Add* button.



- *Continuous* - In this mode, the measurement will not stop automatically unless the *Stop* button is pressed.



- All types of measurements can be started by pressing the *Start* button, but only the *Continuous* measurement requires a manual stopping, all others will be automatically completed.
- Except for the *Single* measurement, it is possible to press *Pause*, which means that no new measurement will be started after the current measurement until it is manually enabled by pressing the *Resume* button (i.e. the current measurement will be completed, but no new measurement will be started).
- *Start button*: Starts the chosen measurement.
- *Live* button switches the DHM in live mode. In this mode the main window displays the measured holograms real-time without any processing. This mode can be used to test the device state: e.g. is there sample in the flow-through cell; the concentration of the actual sample is sufficient; etc.
- *Stop* button: Interrupt and finish the actual measurement or the live mode.
- To help the calibration and test if the DHM working properly, there are further tasks that can be called here:



- *New Hologram*: a new hologram can be taken without starting a complete measurement process. It can be used to test individual holograms. For example, to

estimate the concentration of the sample and allow manual intervention: set the proper dilution, before measurements are started. This requires that the system is already filled with the sample. This can be achieved using the *System filling protocol* button.

Calibration of the Fluor camera can be supervised here too. Pressing the *New hologram* button shows a window, where the overlapped holographic and fluorescent images are shown using the actual calibration based transformation (see Calibration session). If it is correct, then there is no need for further calibration (autocalibration) and the measurement can be started.

- *Background*: Shows the background. It provides information about the cleanliness of the measuring cuvette. If the background appears too cloudy, or stained, speckled, it is worth applying a proper cleaning process (see cleaning protocol) or ultimately replacing the cuvette.

Protocols

- *System Filling Protocol*

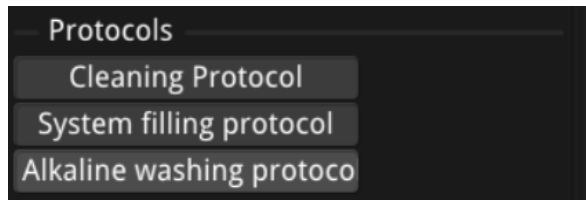
To fill up the tubing and the flow-through cell we have to pump the sample from the sample holder. The System Filling Protocol performs this automatically. This protocol is applied at the start of each measurement.

- *Cleaning Protocol*

To clean the tubing and the flow-through cell we can use different cleaning materials. Usually clean or carbonated water can be applied. If by testing the background we have found that the walls of the flow-through cell are contaminated, specific cleaning solutions (e.g. diluted lye) can be used. After the cleaning process, the system should be filled with clean water.

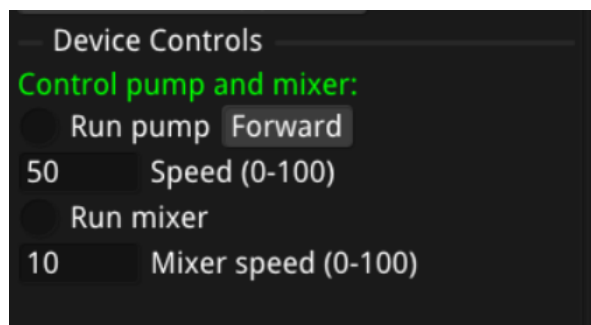
- *Rinsing Protocol*

Between the experiments the previous sample has to be removed from the tubing and the flow-through cell. This can be done by transfusing water through the system.



Device Controls

- *Run pump*: The pump speed can be adjusted on a scale from 0 to 100, with a default value of 50.
- *Run mixer*: The system contains a built-in magnetic stirrer to prevent sample sedimentation during measurements. Without it, samples from the same sources may show different measured densities, due to the precipitation, sedimentation process. The rotation speed is adjustable on a scale from 0 to 100, with a default value of 10. The user can use his(her) own stirrer to ensure the homogeneity of the sample during the measurement.



Plot Page

This page shows object concentration per measurement over time. Each point represents a whole measurement, the number of objects in each class. Moving average is calculated between measurement cycles if “Continuous”, “Interval” or “Sequence” measurements are performed. It is possible to regenerate the moving average values at any time with parameterized breaks between measurements. This page shows only the current Microscope output directory. At the end of each measurement this page is refreshed with the new data.

Features:

- Edit >> Clear plots, empty current plots (does not delete the data)
- Edit >> Reload cache, reloads the saved plot data (useful to revert Clear plots)
- Edit >> Recalculate cache >> Clear the plots and start a query on the measurements to recalculate the plot data using optional “Breakpoint guessing” (It will add a breakpoint between measurements, where time difference is greater than specified).

Breakpoint is inserted between non-connected measurements.

You can select switch classes you want to display under the menu Classes option.

Under “Preferences”, you can choose what data you want to display.

Under “Navigate” you can set the time axis properties.

“Realtime” option will keep the time axis on the current time.

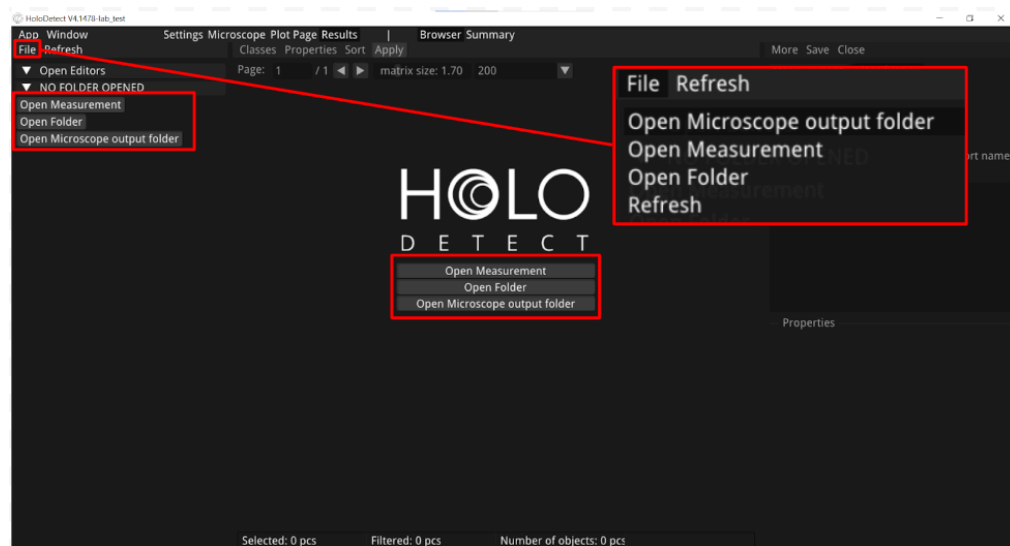
Results

The results of the measurement can be analyzed in two different windows:

You can either view the reconstructed images of the measured cells in a built-in *Browser*, or you can view the *Summary* of the measurement, where the classification results are shown in tables and on charts and the distribution of some of the interesting, measured features are shown on graphs.

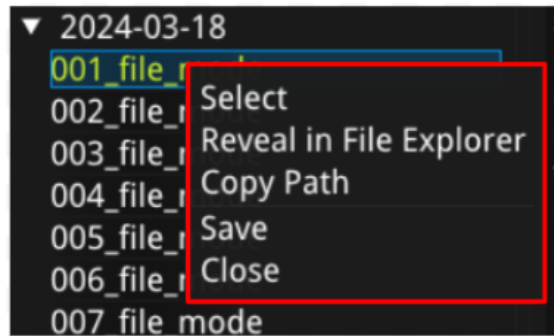
Browser

The objects found and classified in the samples are displayed here. There are three options for opening folders and measurements: with *Open Folder/ Measurement* you can open any measurement folder/measurement, and with *Open Microscope Output Folder* you can open the folder where the current measurement is stored. You can access these buttons in three different ways:

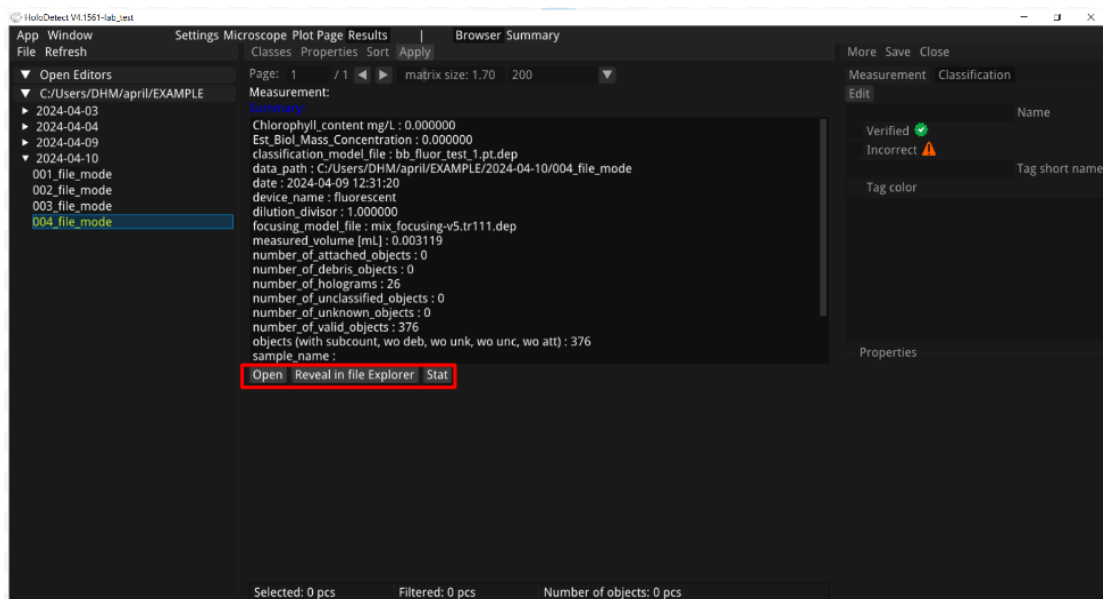


- Clicking on the *Open Microscope output folder* button will open the same folder as the one specified in the *Settings* panel).
- By clicking on the *Open Folder* button, the pop-up window will allow you to freely choose a folder.
- By clicking on the *Open Measurement* button, the pop-up window will allow you to freely choose a measurement.
- The days on which measurements were taken will then be displayed, and the measurement folders can be accessed by clicking on the arrows next to them. If you

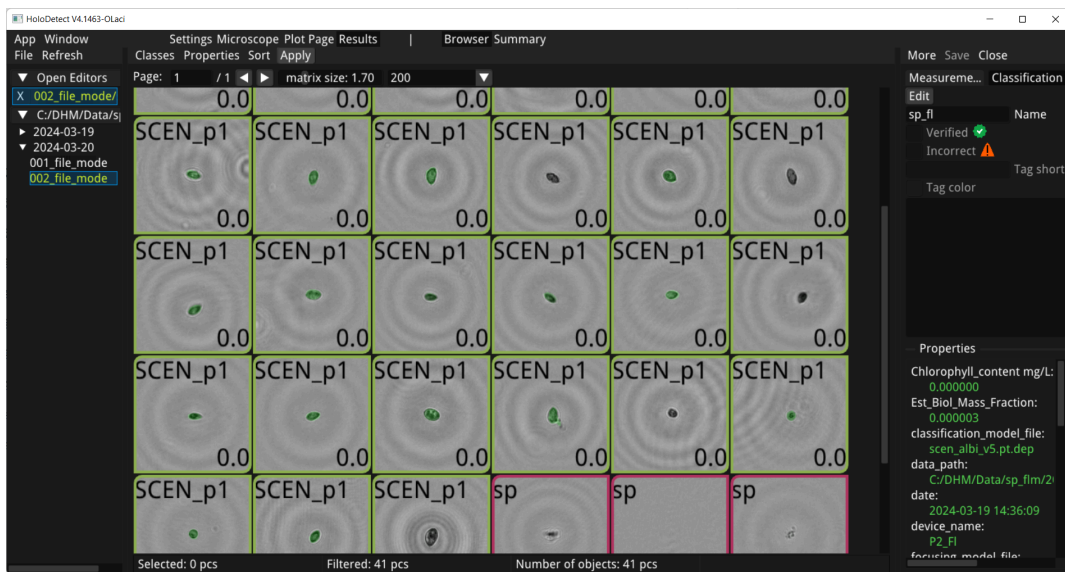
move the mouse over them, you can see their paths, and right-clicking on them will bring up several options: *Select* to select and open the measurement folders, *Reveal in File Explorer* to open the measurement folder outside the program, *Copy Path* to copy the path, *Save* to save changes, or *Close* to close.



- If a measurement is selected (i.e. clicked once) but not yet opened, all related information is displayed.

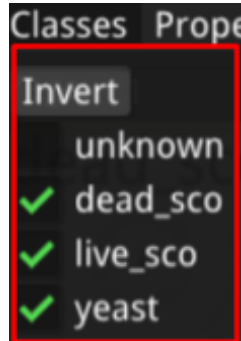


- There are three buttons to choose from: open the measurement with the *Open* button, open the measurement folder in the Windows Explorer with the *Reveal in file Explorer* button, and open the measurement statistics by clicking on the *Stat* button.
- Select a measurement and open it with the *Open* button.

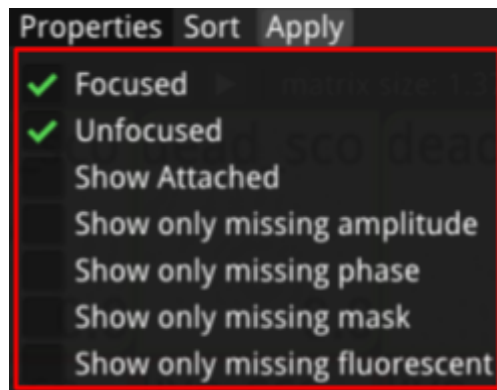


- The objects in the sample are displayed, sorted by their classes. At the top of the page, you can set the size of the matrix to be displayed by setting the Matrix size to (default value 1.70), or you can set the number of images to be displayed per page from 100 to 1000 (default value 200).

- You can filter the images you want to display according to various criteria.
 - It is possible to display only objects belonging to a certain class by clicking on the Classes button and selecting the relevant class(es).

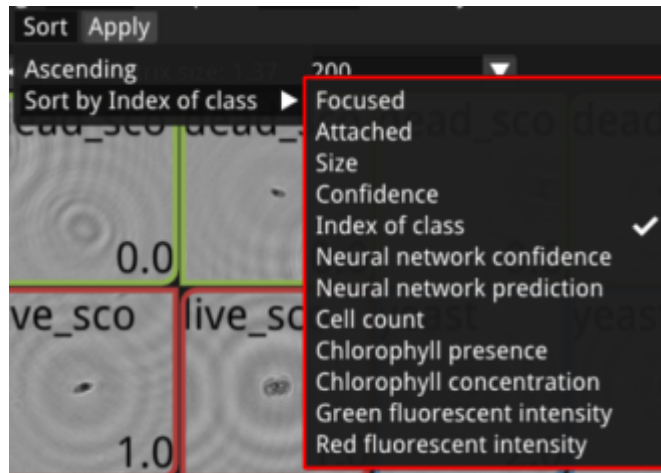


- Properties (by default, Focused and Unfocused objects are displayed)



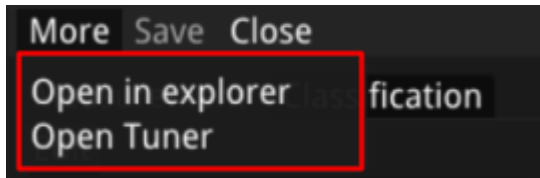
- *Focused*: only focused objects are displayed.
- *Unfocused*: only images that are out of focus are displayed.
- *Show Attached*: displays objects stuck to the wall of the measuring cell.
- *Show only missing amplitude*: It will only display objects that are missing their amplitude image (amplitude is the image that basically appears in the results).
- *Show only missing phase*: displays objects that do not have a phase image due to some error
- *Show only missing mask*: displays objects that do not have a mask image due to some error
- *Show only missing fluorescent*: displays objects that do not have a fluorescent image due to some error

- Press the *Sort* button to sort the objects by 12 different parameters.

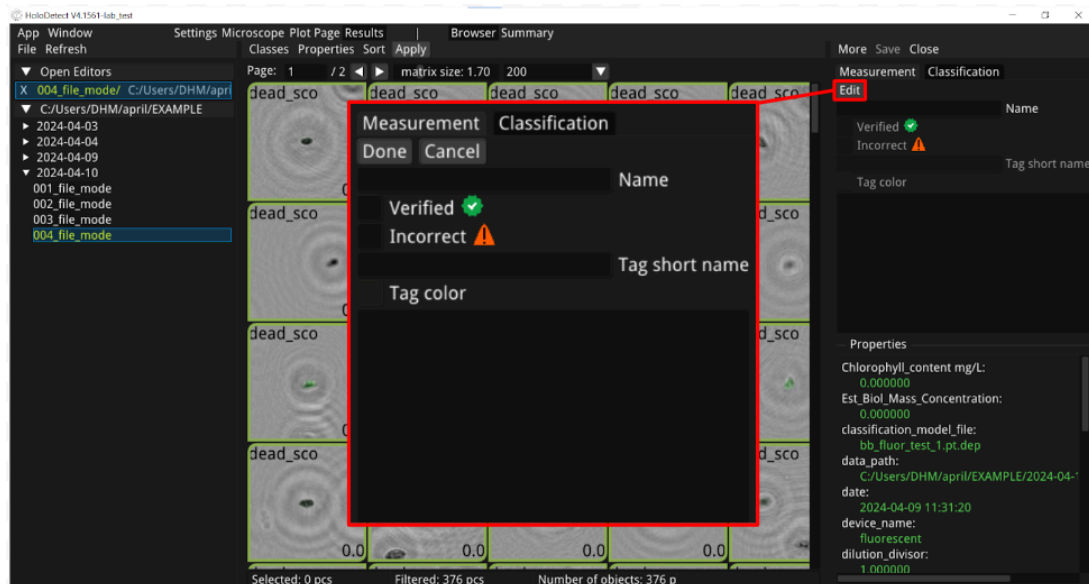


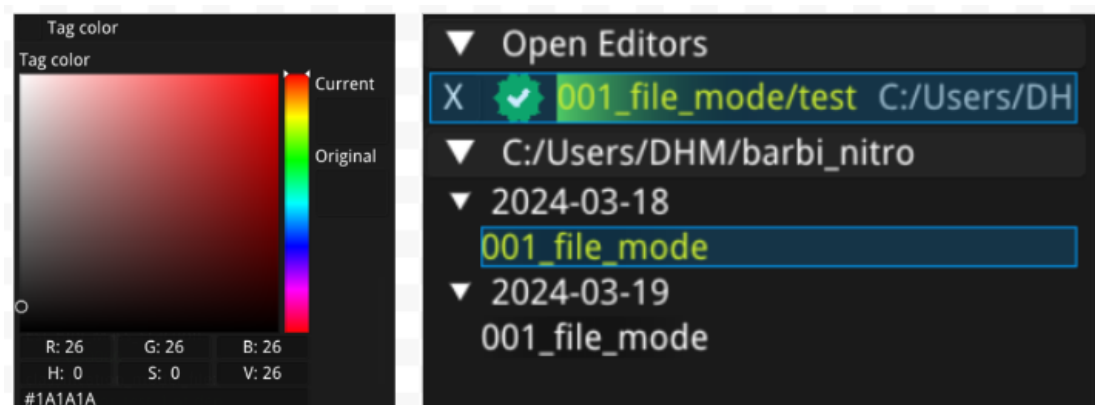
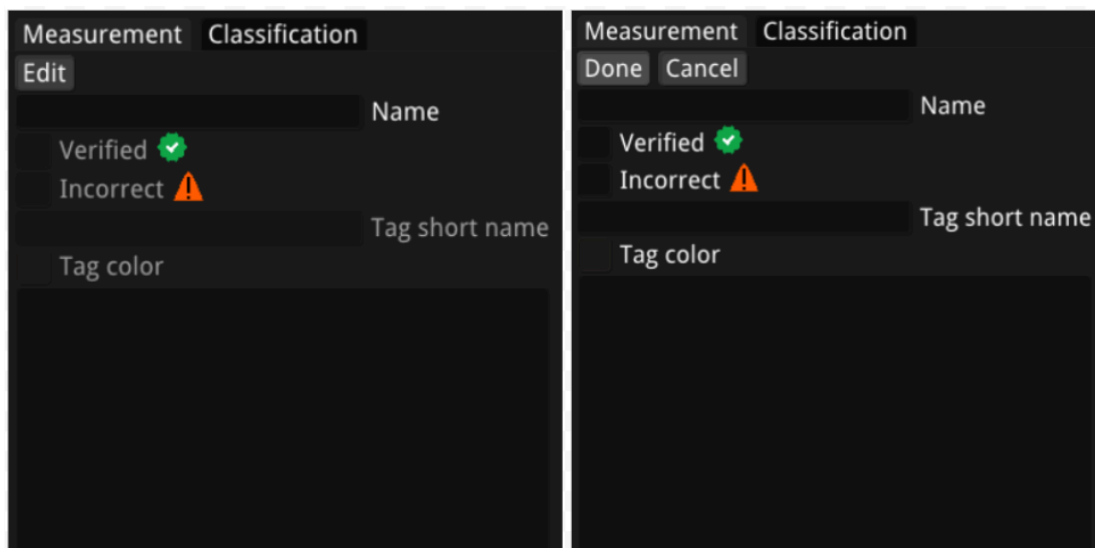
- *Focused*: Indicates whether the object is in focus or not.
- *Attached*: Indicates whether or not the object is attached to the wall of the measurement cell.
- *Size*: Specifies the estimated size of the object in pixels.
- *Confidence*: Indicates how confident the neural network is in the classification on a 0-1 scale. Can be manually modified afterwards.
- *Index of class*: Each class is assigned a number, which is used to sort the objects. Can be manually modified afterwards.
- *Neural network confidence*: Indicates how confident the neural network is in the classification on a 0-1 scale. Cannot be manually modified afterwards.
- *Neural network prediction*: Each class is assigned a number, which is used to sort the objects. Can be manually modified afterwards.
- *Cell count*: Estimated number of cells in the image.
- *Chlorophyll presence*: true or false depending on the chlorophyll content of the cell (above or below a threshold)
- *Chlorophyll content*: the estimated chlorophyll concentration of the actual cell (μg).
- *Green fluorescent intensity*: The average measured fluorescence in the segmented object area.
- *Red fluorescent intensity*: The average measured fluorescence in the segmented object area.

- After selecting the filter parameter, click *Apply* to display the filtered images.
- Clicking on the *More* button in the top right corner allows you to open the measurement outside the program (*Open in explorer*), or to open the Few-Shot Tuner window (*Open Tuner* - more on this later).



- By pressing *Save*, you can save the changes you have made to a measurement, and by pressing *Close*, you can close it (if another measurement is open, the view automatically switches to that one).
- By clicking on the *Edit* button under the *Measurement* tab, you can change the name of the measurement, you can tag it as *Verified* or *Incorrect* (these will appear as a green tick and an orange triangle next to the measurement name in the top left corner), and using *Tag short name* you can give the measurement a short name (6 characters), which will help you to distinguish the measurements from each other in the future. *Tag color* allows you to tag the measurements (the color will appear in the upper left corner under *Open Editors* next to the measurement name).






- There is also a text box where you can describe each measurement in more detail. Here you will also find some important information about the measurements (the same information is displayed when you select a measurement before opening it). To modify the *Name*, *Verified*, *Incorrect*, *Tag short name*, *Tag color* parameters and description, always click *Edit* first. To complete the changes, click *Done* first and *Save* to save them.


More Save Close

Measurement Classification

Done Cancel

Name

Verified 

Incorrect 

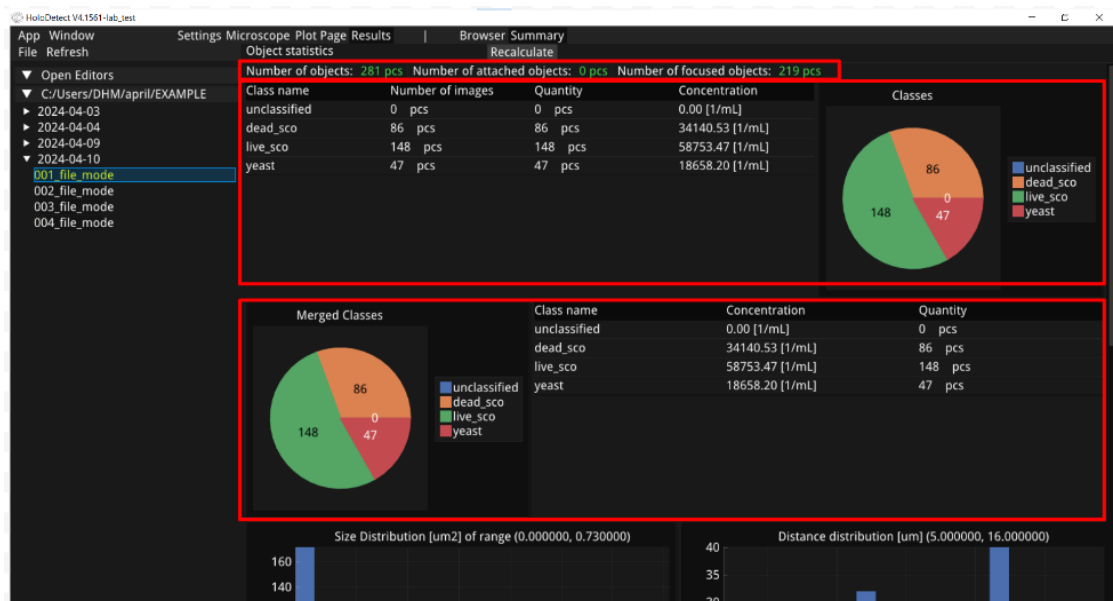
Tag short name

Tag color

Here you can describe the measurement
by clicking on the Edit button

Summary

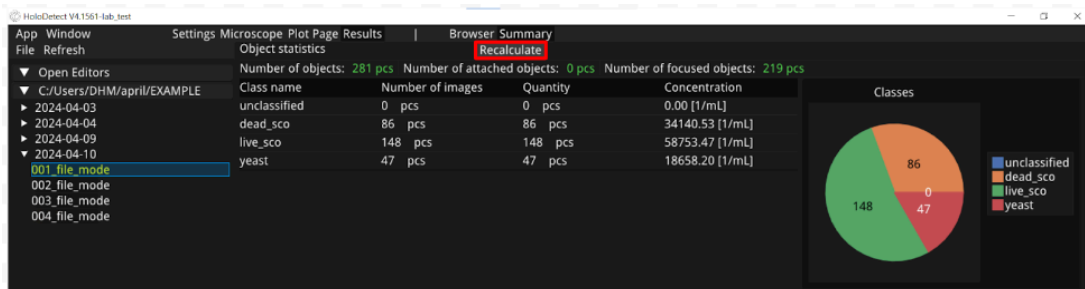
- Here you can find the statistics for the measurement. The top row shows how many objects the program has found in the sample, how many of them were adhering to the surface (which are not taken into account when calculating the statistics) and how many of the remaining objects it evaluates as being in focus. It is possible to change the *Focused* and *Attached* parameters manually after opening the object in the *Browser* page.



- The page contains two tables:
 - The first table and pie chart show the distribution of each separate class. Under the Number of images column is the number of images in each class, while under the Quantity column is the sub-count assigned to each object in the summation.
 - The second table and pie chart show the merged classes. The software can merge classes and thus automatically calculates how many cells were included in total. To do this, you must follow the rules of nomenclature: class_name_p, class_name_p2, class_name_p3. To merge them at the end, the class name must be the same everywhere and each must be followed by a p and a number, for example if you want to classify Scenedesmus, you can use the classes SCEN_p1, SCEN_p2 and SCEN_p3. These will appear as SCENs in the second table and will contain all the cells from the listed

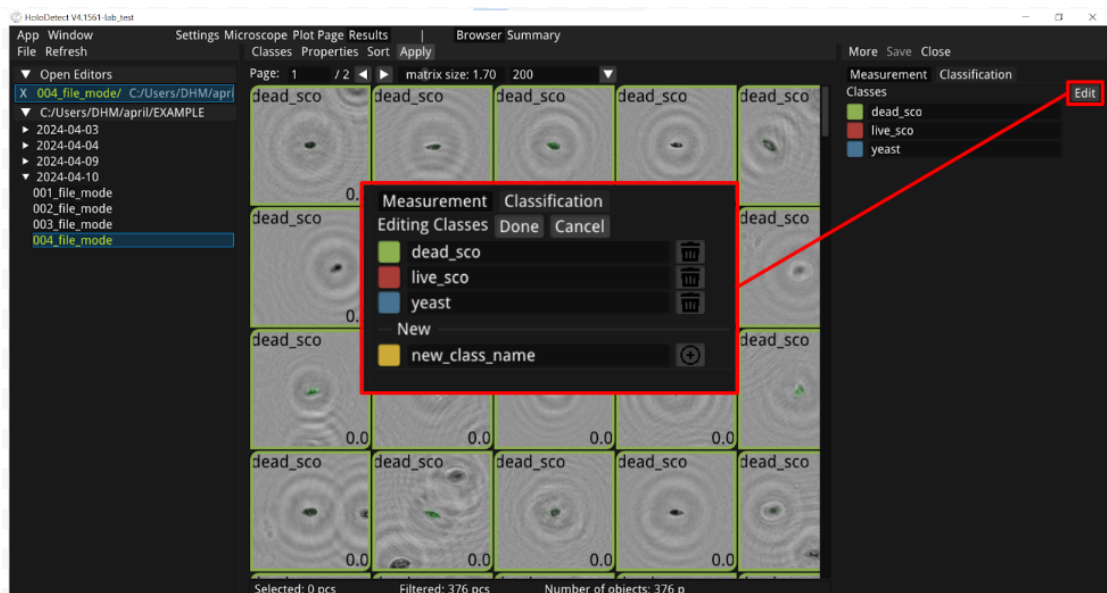
classes. The summation is based on the sub-counts, which can be modified if the neural network did not accurately estimate the cell count. (Such classes are best created when working with a sample that typically contains clumped cells. In this case, the class SCEN_p1 may contain single cells, SCEN_p2 may contain cells clustered by twos, and SCEN_p3 may contain cells clustered by threes.)

- There are three bar charts at the bottom of the page:
 - The first represents the size distribution (in pixels) of the objects found in the measurement.
 - The second shows the distribution of the reconstruction depths (in micrometers) of the objects found in the measurement.
 - The third shows the distribution of the measured fluorescence intensities of the objects of the measurement in the different color channels.
- If there has been a change in the Browser panel that has affected the classification, the Recalculate button can be used to update the statistics for the measurement.

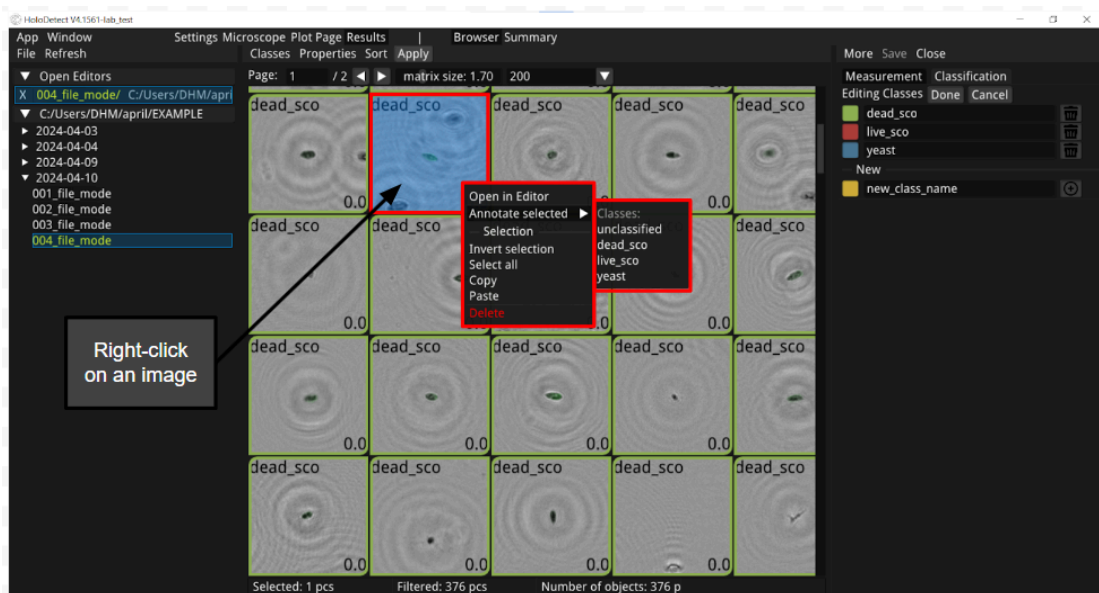


Classification

- Next to the *Measurement* page is the *Classification* page, where you can find the classes used in the classification with their corresponding colors.
- By clicking on the *Edit* button, you can modify them in three ways: delete existing classes, add new class(es) or rename class(es). If you delete an existing class, the objects belonging to that class will be moved to the so-called *Unknown* class (*unclassified*) but will not be deleted from the measurement. A new class can be added by clicking on the “+” button. To rename an existing class, click on the relevant line and enter the desired name. To save your changes, first click *Done*, then *Save*.

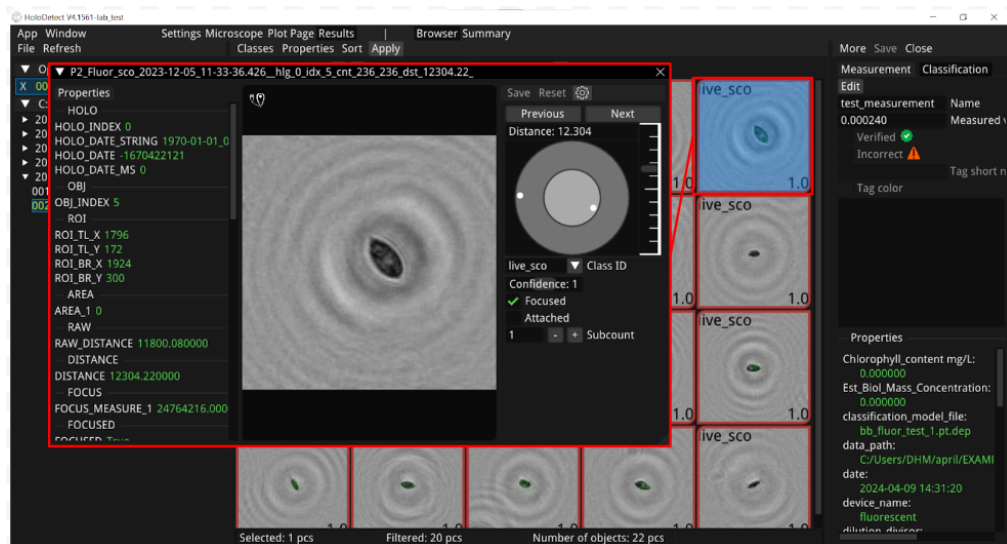


- Right-click on any object and a list of possible activities will be displayed.
 - Click on the *Open in Editor* button to open the image.
 - You can reclassify it using the *Annotate selected* button.
 - Press the *Invert selection* button to invert the selection.
 - You can select all objects by pressing the *Select all* button.
 - With the *Copy* and *Paste* buttons it is possible to copy the selected objects from one measurement to another. To do this, first select the objects you want to copy from one of the measurements, press the *Copy* button, then open the other folder and right-click on the *Paste* option.
 - You can also delete an image(s) by pressing the *Delete* button.



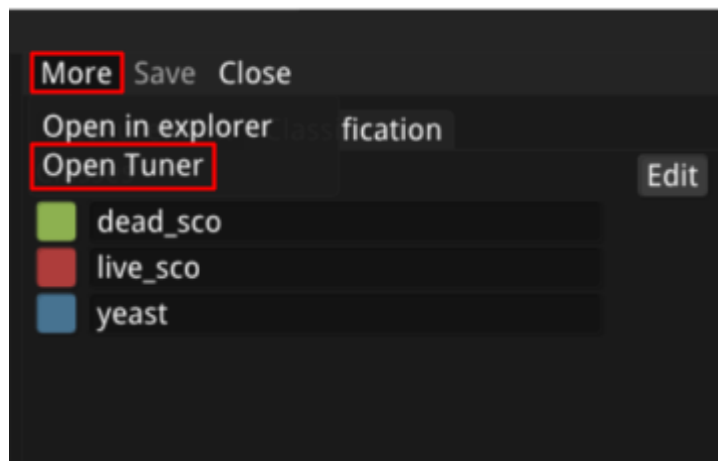
- You can also use the Ctrl+A (*Select all*), Ctrl+C (*Copy*), Ctrl+V (*Paste*) and Ctrl+S (*Save*) keyboard shortcuts.
- Open an image - double-click on a selected image or right-click and select *Open in Editor*.
 - On the left side you can find information about the image, and on the right side you have the possibility to modify some of its properties. The *Previous* and *Next* buttons allow you to move between images, and the focusing knob underneath adjusts the focus. Here you will also find the *Class ID*, which is the class to which the object has been assigned, this can be changed using the drop-down list. It is also possible to change the *Confidence*, which allows you to specify how confident you are in the result of the rating (here you can choose between 0,1 and 2, where 0 is not at all confident and 2 is completely confident). You can also change whether the object is marked as *Focused* and whether it is *Attached* to the measuring cell wall. The *sub-count* value specifies the estimated number of cells in the image, which can be changed afterwards.
 - It's important that any changes made in this small window can be saved using the *Save* button at the top of the window, otherwise the changes will be lost.

- You can open more than one window displaying objects at a time, and use the *Next* and *Previous* buttons at the top to move between them.
- Click on the distance meter icon above the image to measure the size of each object. Move the cursor to one point of the object you want to measure, then press and hold the left mouse button until you reach the other point, whose distance you want to measure. When you release the mouse button, the line changes from yellow to green and displays the distance in micrometers between the two points. You can take multiple distance measurements per image. If you want to delete them, you can press the right mouse button to delete them one by one, starting from the last line drawn, but you can press and hold the right mouse button to undo all the lines drawn on the image at once.
- Clicking on the gear icon brings up a list where you can select which images to display in addition to the amplitude. The most important of these are Mask and Fluor, which are set by default to Opacity (the opacity of the layer above the image) of 0.25, and Fluor Chanel, which is set by default to RED.
- In the Focusing block, you can also set the step size of the focusing knob, which by default is 0.010 for Large step and 0.001 for Small step.

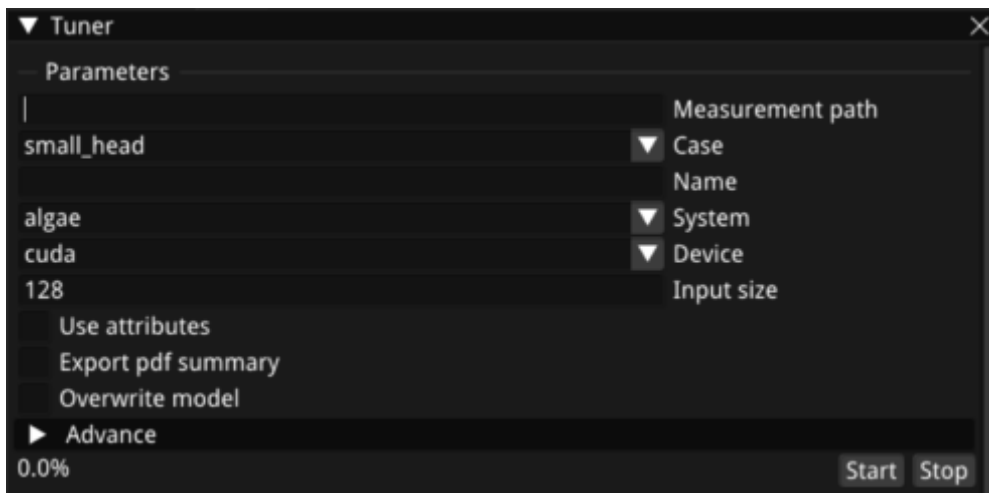


Using the Few-Shot Learning Module

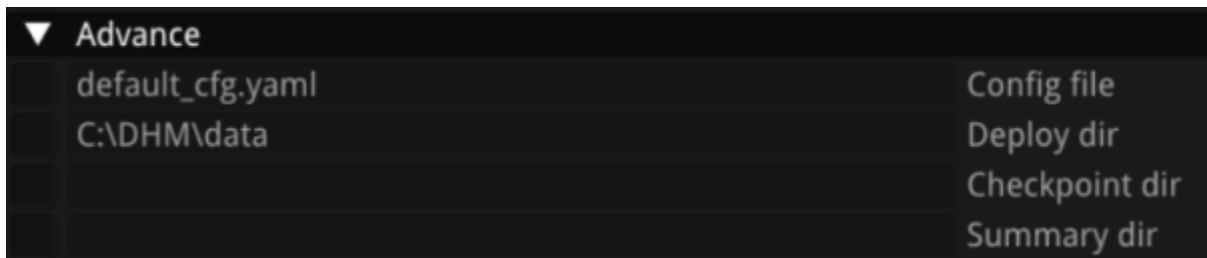
The Few-Shot Tuner can be accessed by clicking the *Tuner* button under the *Window* tab, or by clicking the *More* button in the *Browser* window and selecting *Open Tuner*. To train a new model, you must first specify which measurement folder to use as the training database, this can be set using the *Measurement path* (IMPORTANT! If the Few-Shot Tuner was opened with *Window* → *Tuner*, it will contain the path of the last used folder as *Measurement Path*, BUT if it was opened with *More* → *Open Tuner*, it will automatically fill in the path of the current folder). Before each training session, the measurement folder containing the training database should be saved.



Next, choose one of three *Cases* depending on how much data is available (see Few-Shot Learning Module), and then name the model you are creating. The *System*, *Device* and *Input size* parameters do not need to be changed (default values: *System* - *algae*, *Device* - *cuda*, *Input size* - *128*).



In case you want the fluorescence data to be taken into account in model training, you can do so by selecting *Use attributes*. It is possible to save the results in PDF format by selecting *Export pdf summary*. To do this, click on the *Advance-tab* and select the folder where you want to save the results as *Summary dir* (first you must check the box at the beginning of the row to edit the path to the *Summary* directory). If you have made any changes to the measurement folder you want to train Few-Shot on, **you must save it first!** To start training, press the *Start* button. The training is complete when the screen displays: *Model deployed successfully*.



Once the new model has been successfully created, you can click on the *Refresh Classification model list* button in the *Settings* panel to select the newly created model from the list and start a new measurement, now with this new classifier.

The window can be reduced in size, with the help of the little arrow you can reopen it at any time.

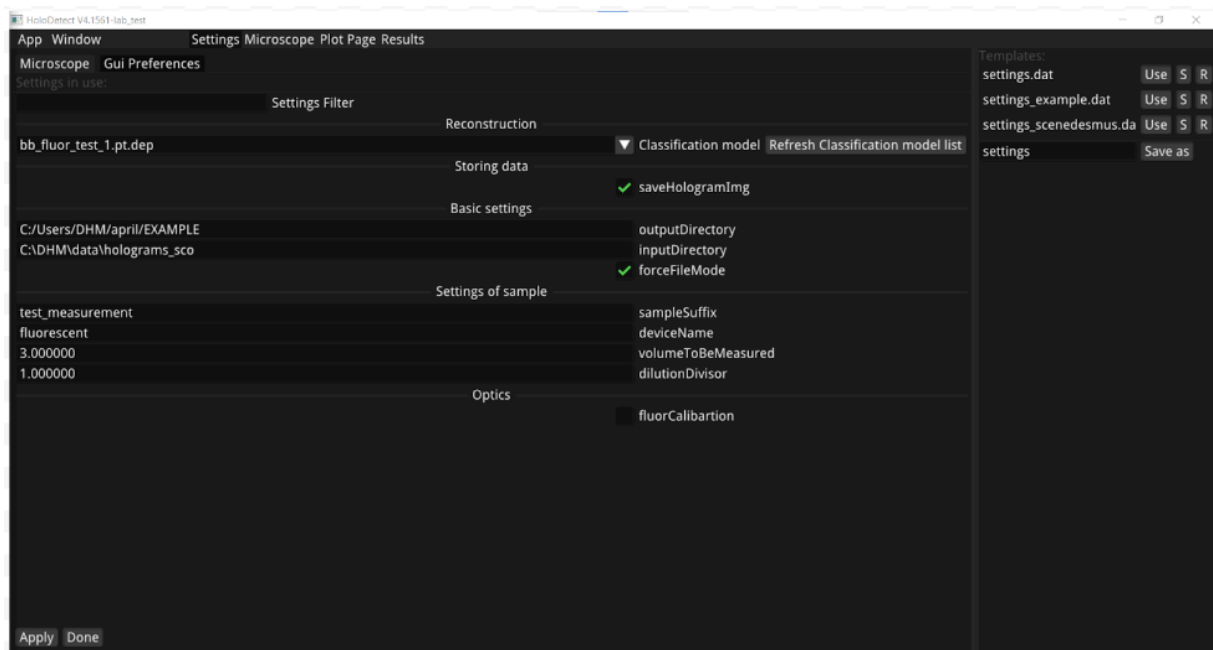


Step-by-step measurement demonstration

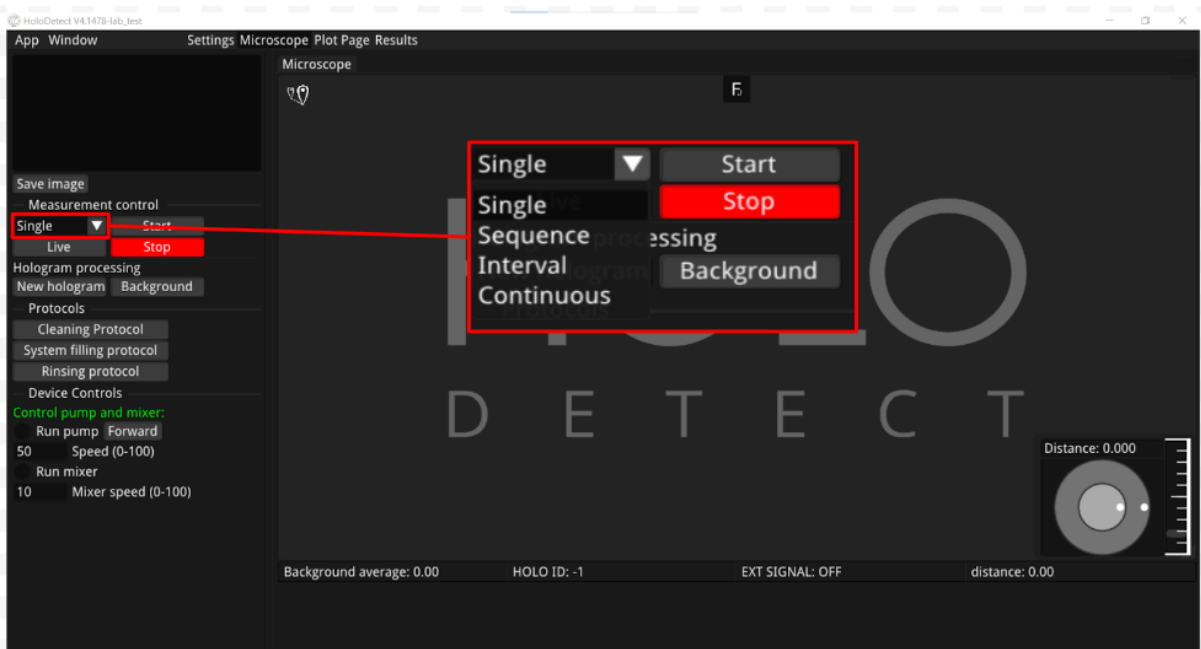
Describing the steps of a measurement, evaluating the results.

First, select one of the Settings files on the right. If available, set a Classification model, then select whether you want the holograms you have created to be saved or not (saveHologramImg). Next, specify the folder where you want to save the results (outputDirectory) and, if you are using File Mode, the folder (inputDirectory) from which you want to reprocess the holograms contained in it. In this case, you should also select forceFileMode. Give a name to the measurement (sampleSuffix), name the device (deviceName) and then set the volume to be measured (volumeToBeMeasured). Finally, enter the dilution rate if you want to get the results corrected for this. If you want to use this Settings file in the future, click Save as on the right side and enter a name of your choice.

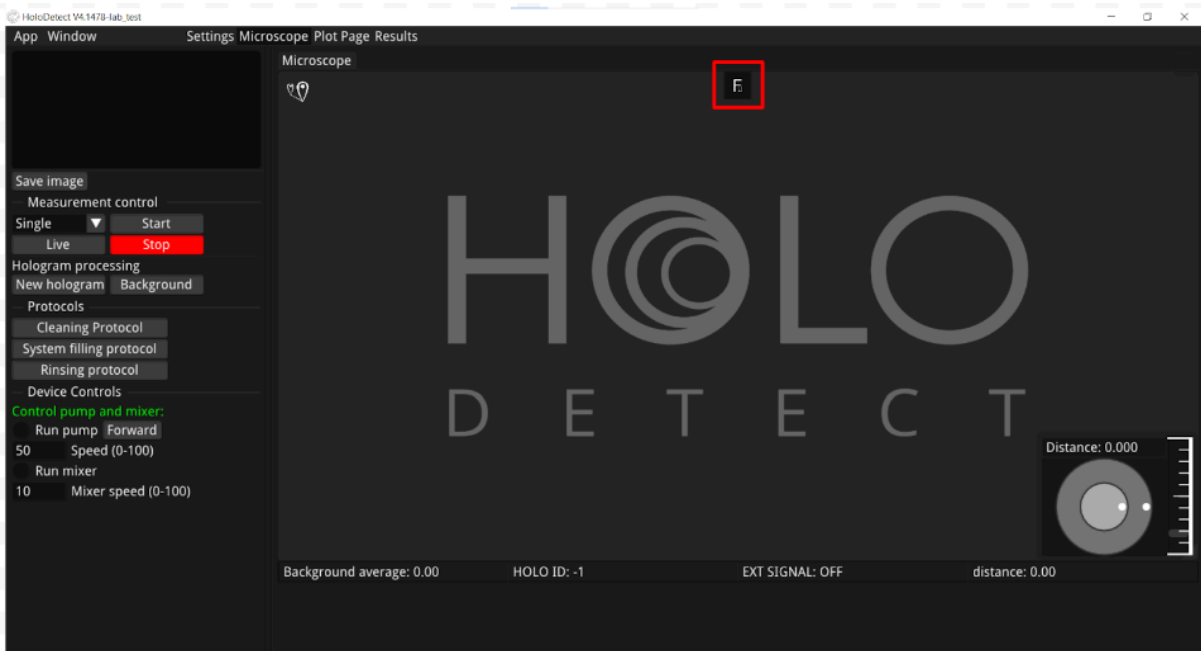
There are several buttons to the right of the Settings files: click on Use to open the file you have selected, click on S to save it, click on R to rename it, click on D to duplicate it and click on X to delete it.

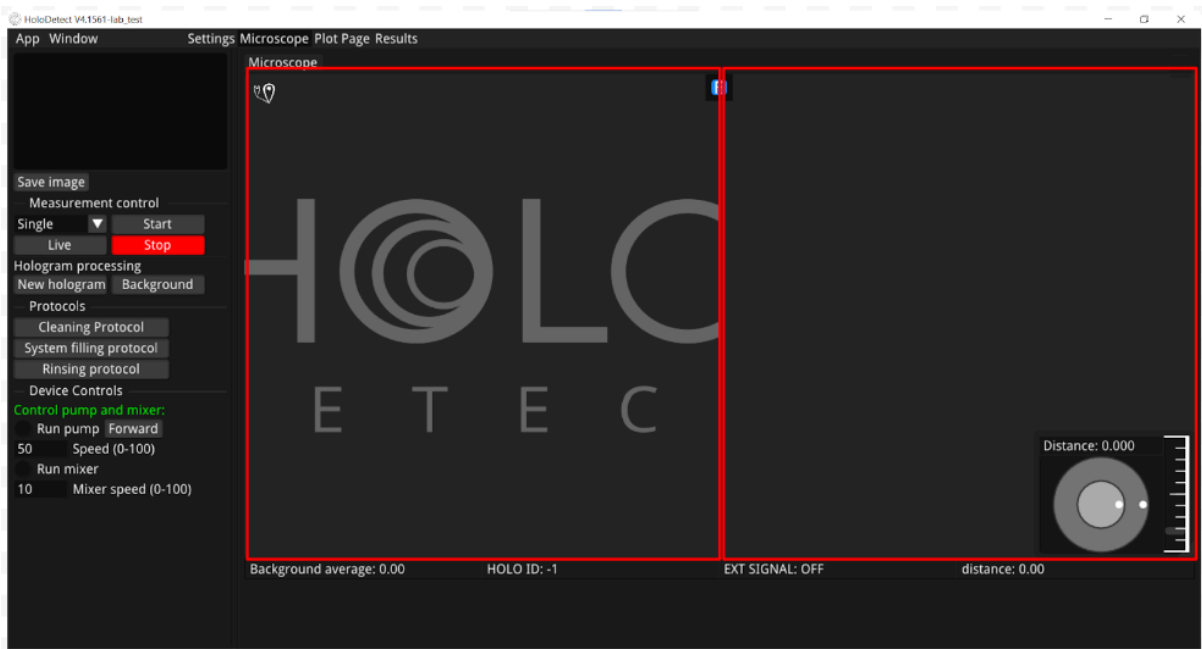


For measurements, you can choose from the four options already mentioned: Single, Sequence, Interval and Continuous.

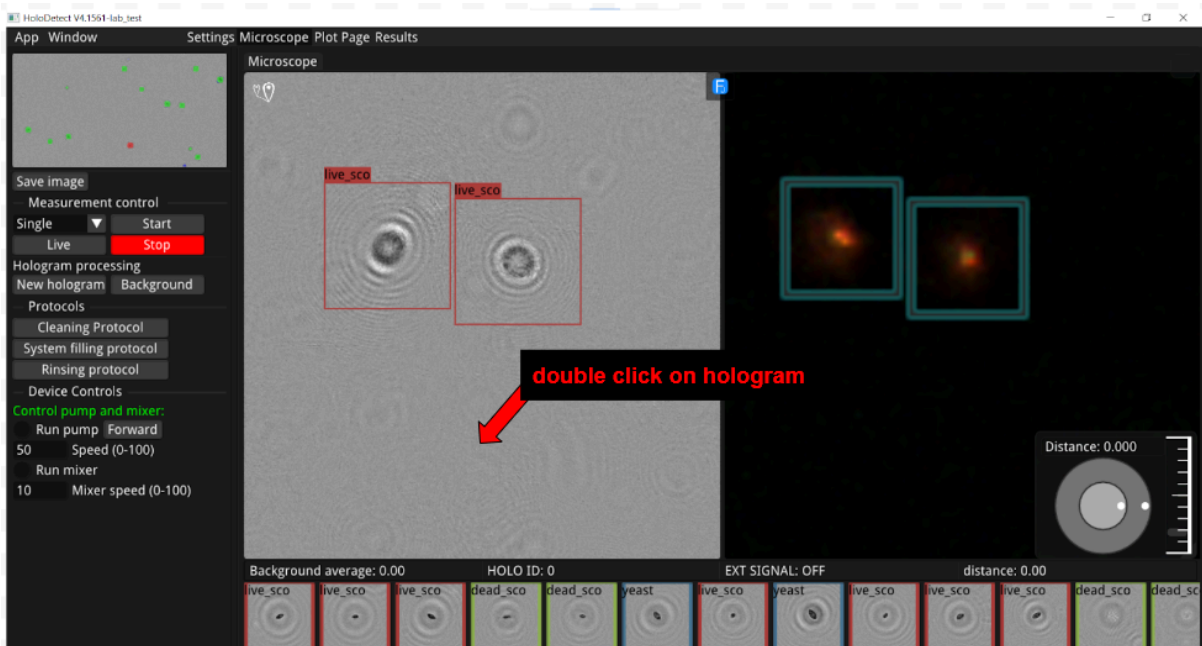


Press the Start button to start the measurement, then click the F button in the middle of the screen to display the fluorescence image and the hologram at the same time.



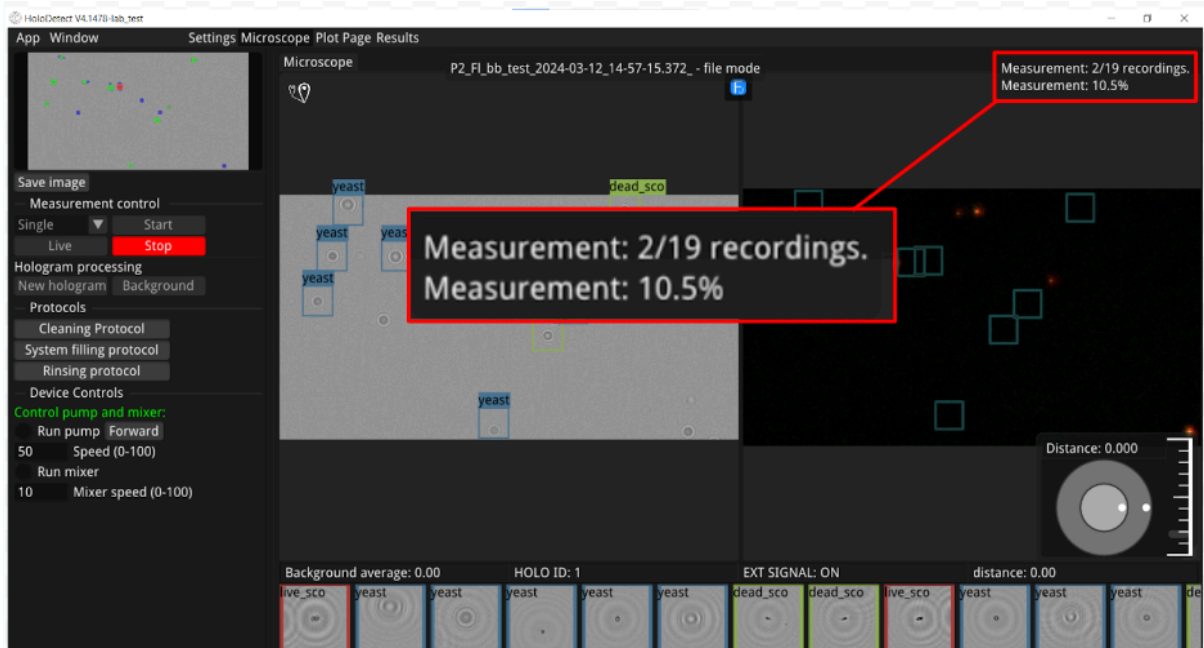


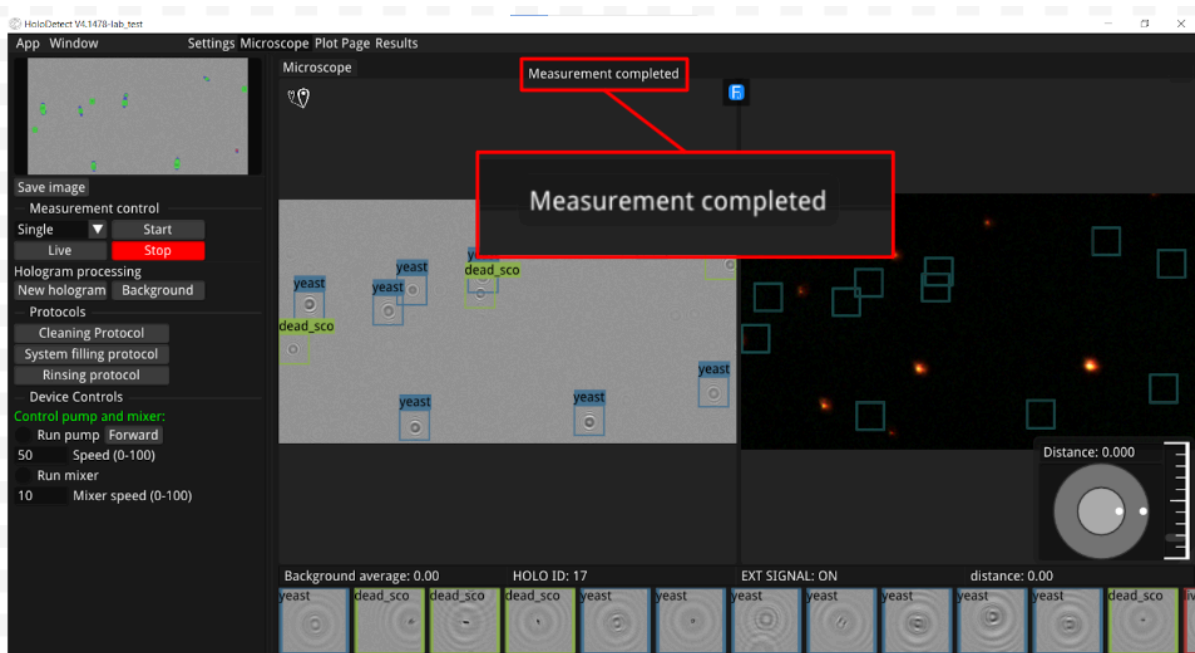
Double-click on the holographic image to display both the holographic and fluorescence images at full size.



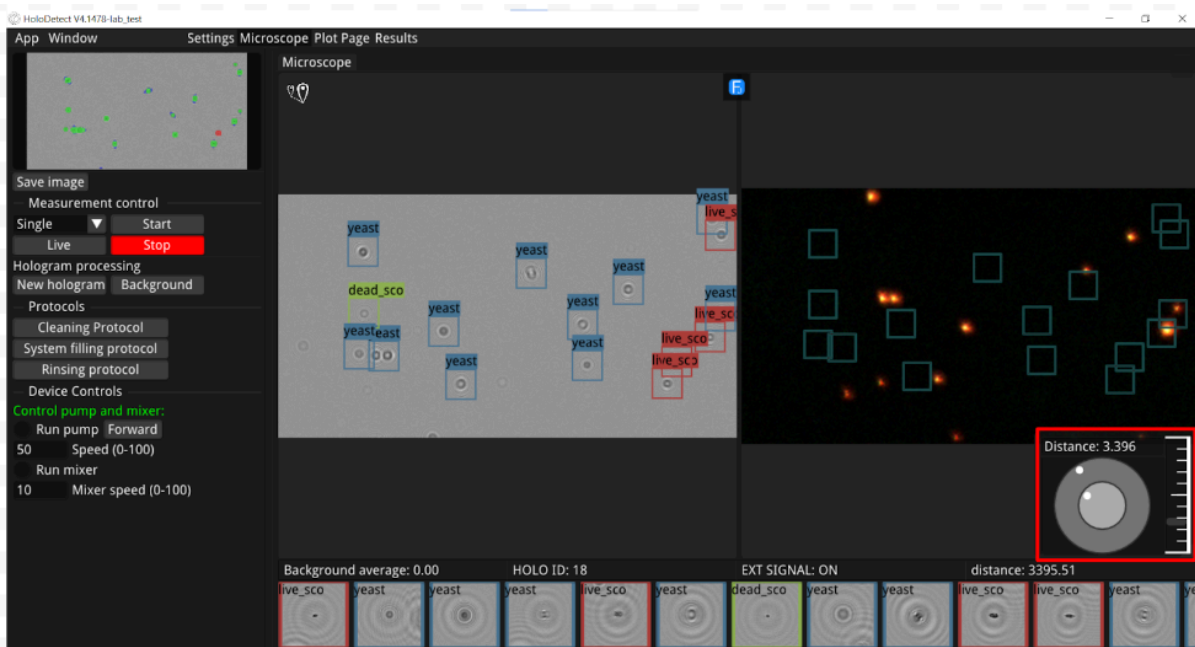


At the top right of the screen you can see the status of the measurement, and when it is complete, you will see the text 'Measurement completed' in the middle of the screen.

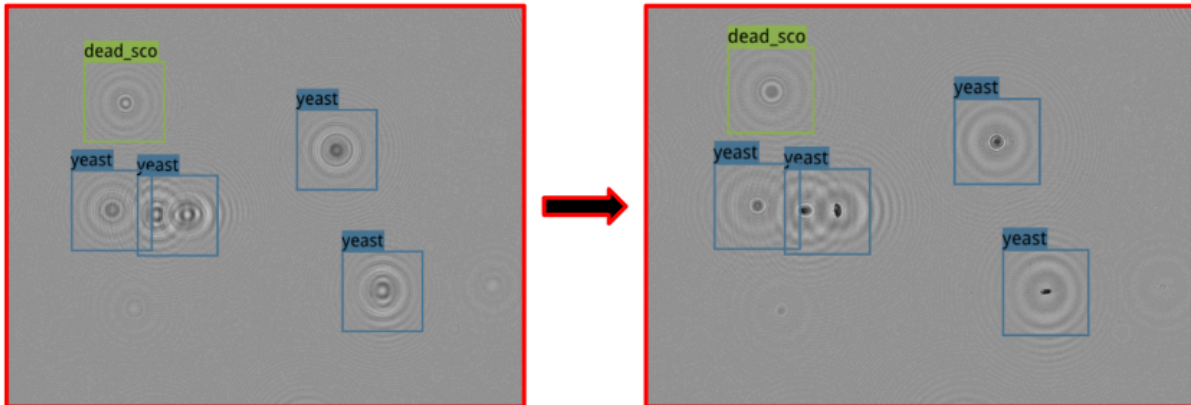




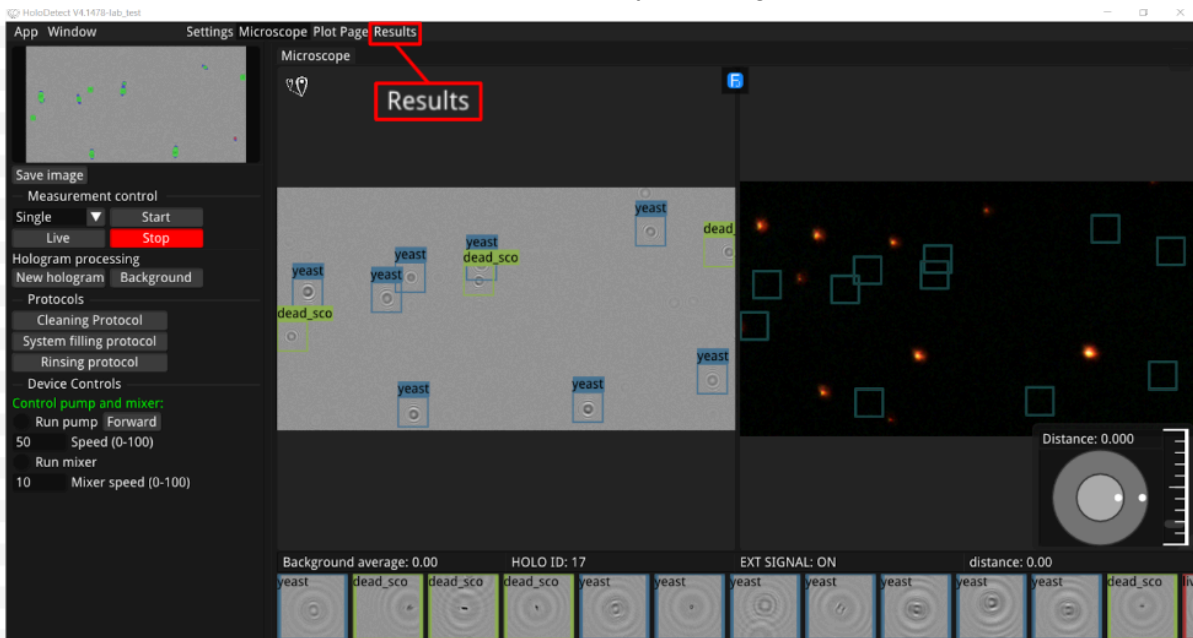
When you click on the New hologram button, stop a measurement in progress, or a measurement is finished and the last image is open, you have the option to focus the hologram or save the original or the refocused hologram by clicking on the Save image button.



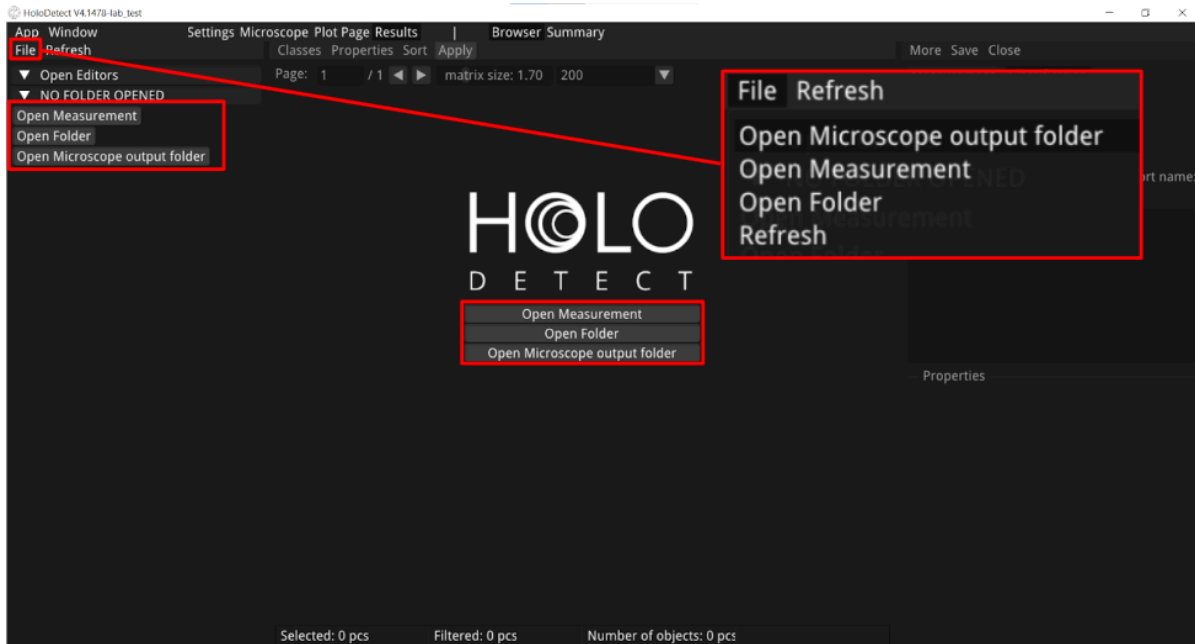
Focusing a hologram:



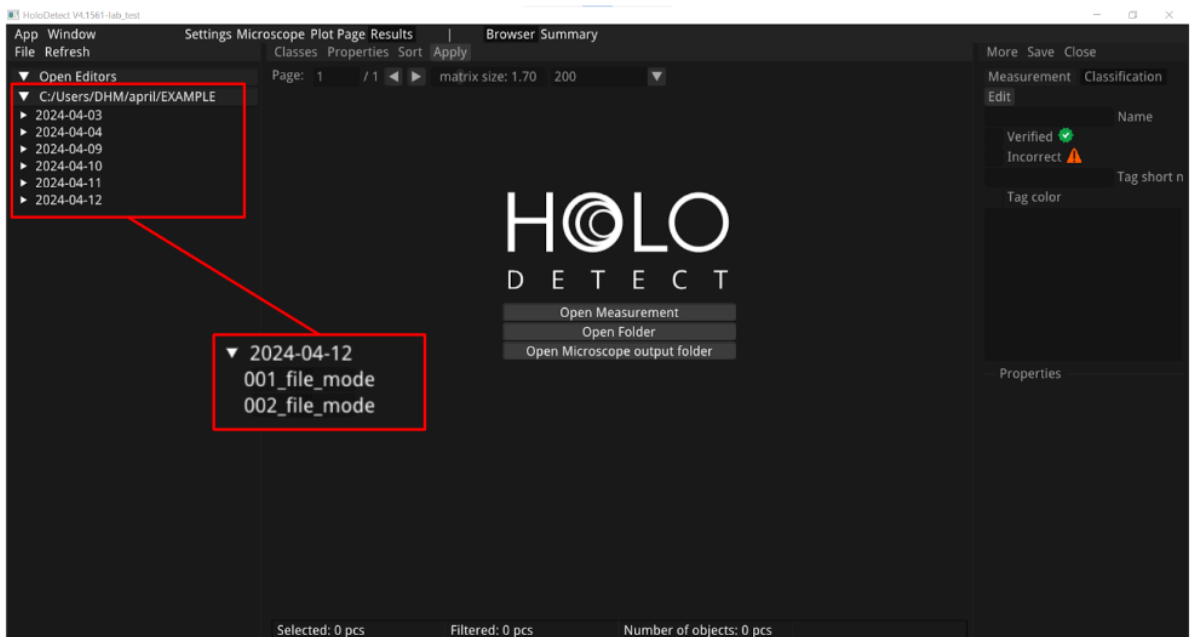
You can view the results for each measurement by pressing the Results button.

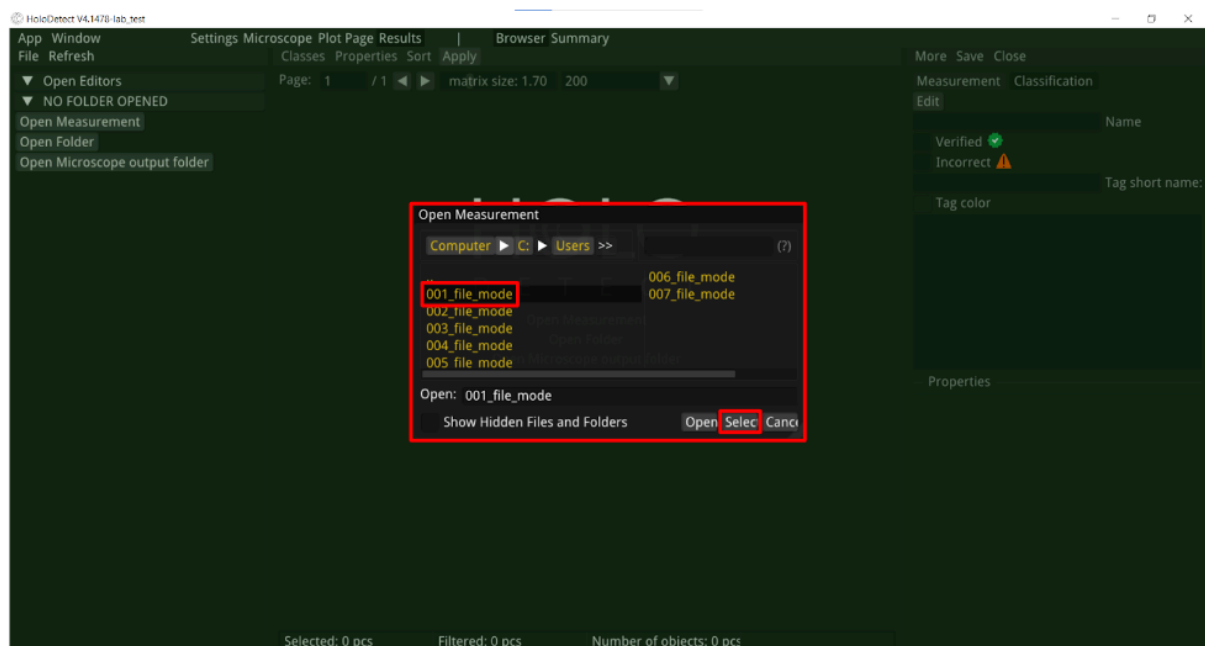
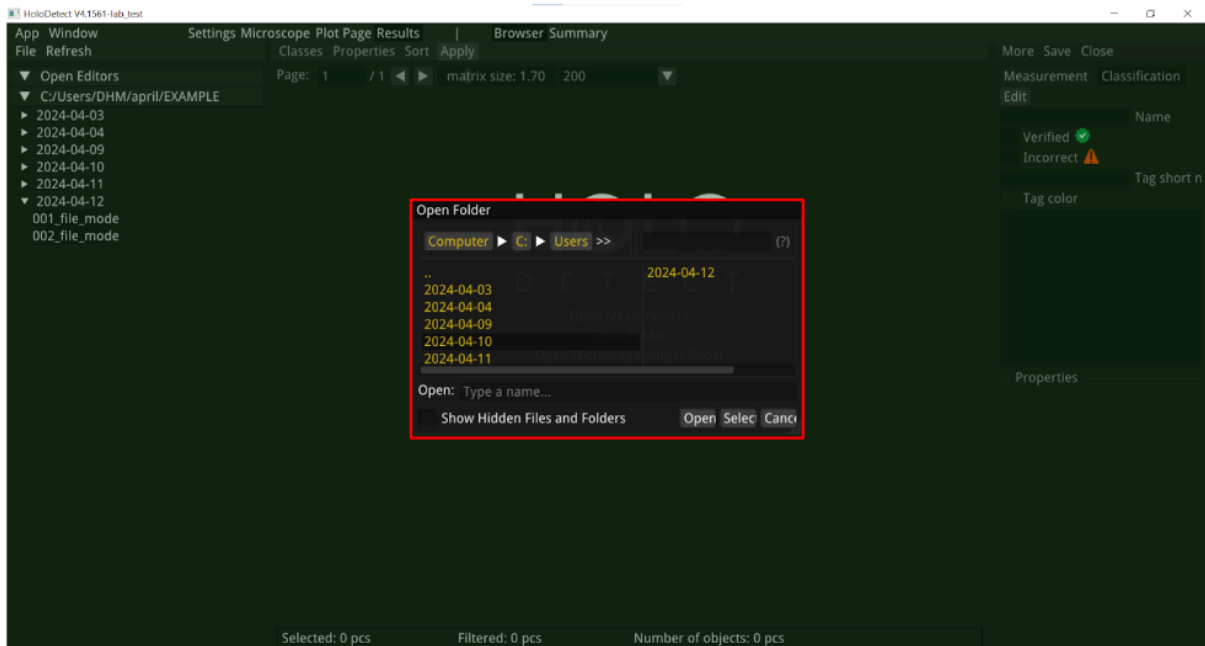


There are several ways to open the measurements: you can select the folder you specified in the Settings (Open Microscope output folder), or you can open a separate measurement (Open Measurement) or a folder of your choice (Open Folder).



Select the measurement you want to open from the list on the left.

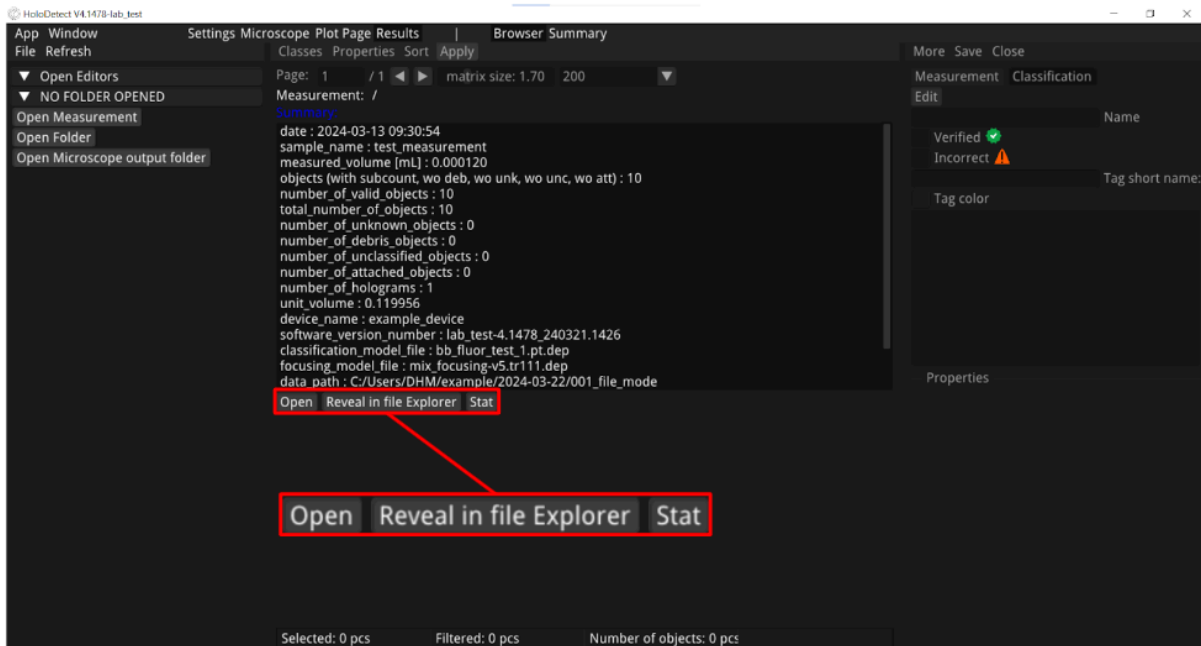




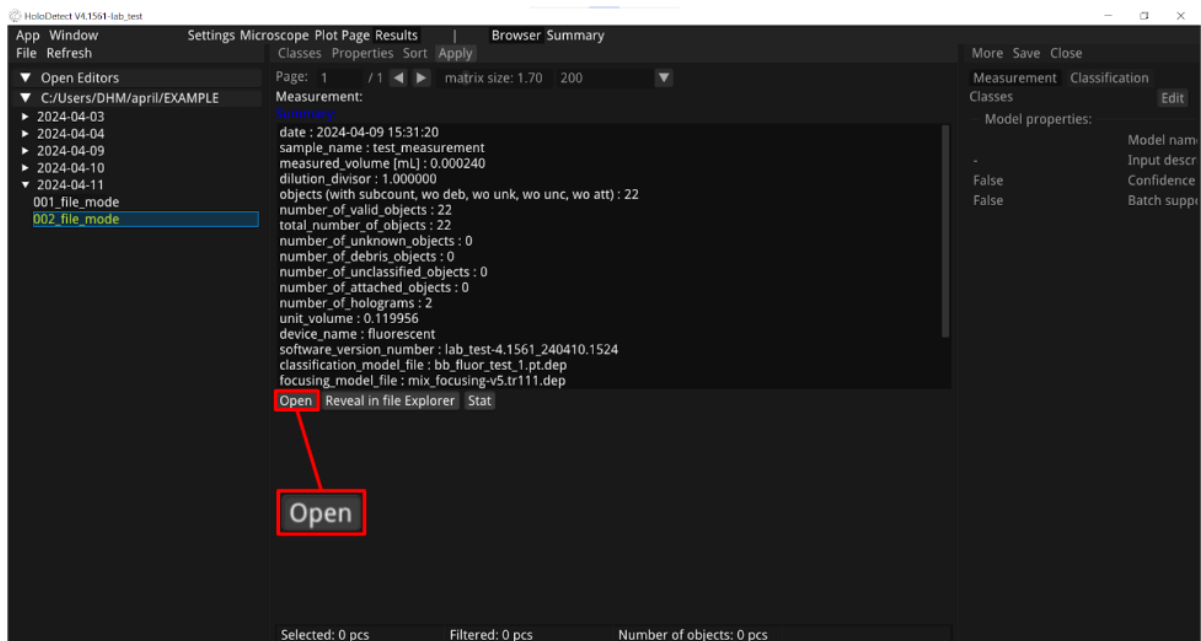
Once you click on it, you have three options to choose from:

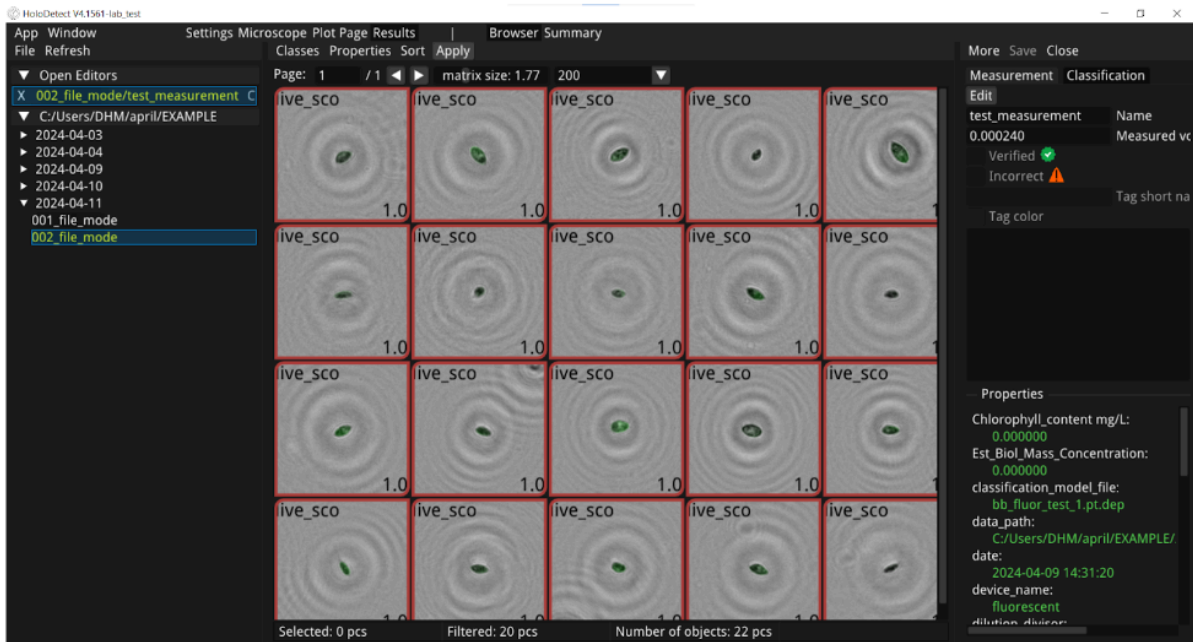
1. open the measurement (Open)
2. open it in File Explorer (Reveal in file Explorer)
3. or open the statistics for the measurement (Stat).

If you double-click on the selected measurement, it will open right away.

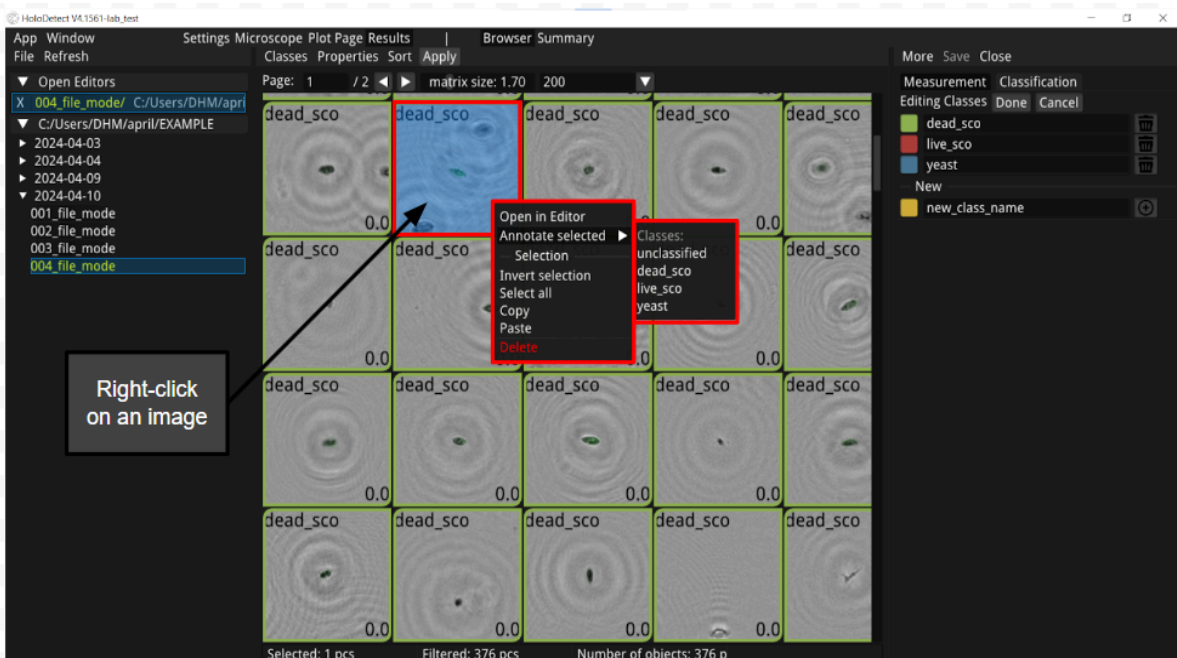


To view the measurement, select Open.

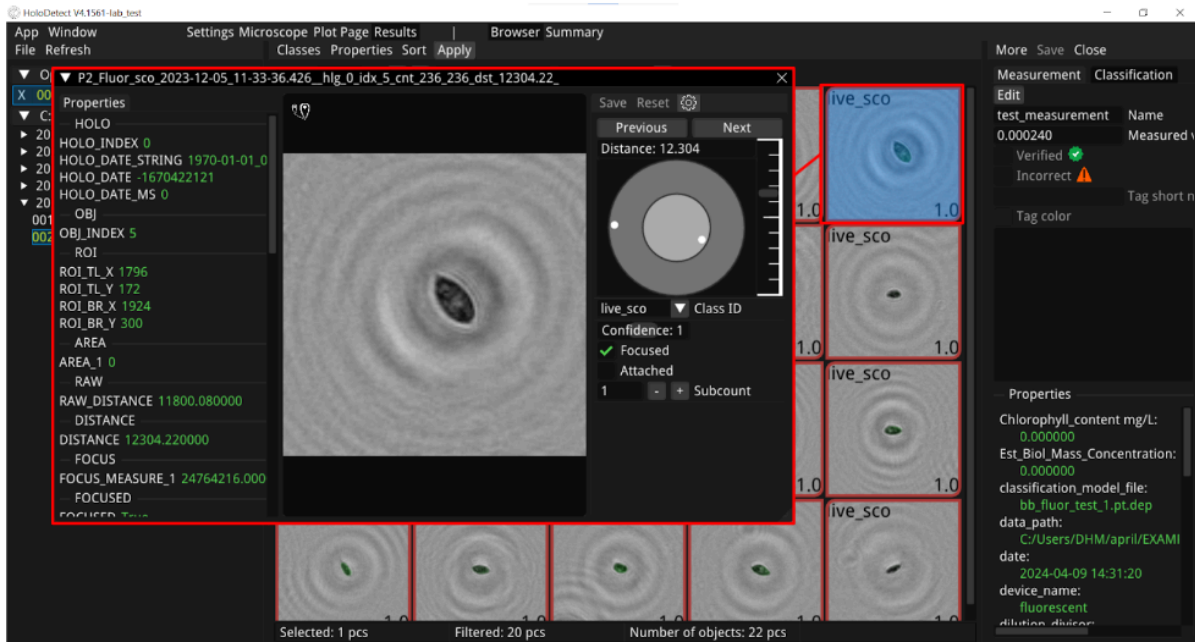




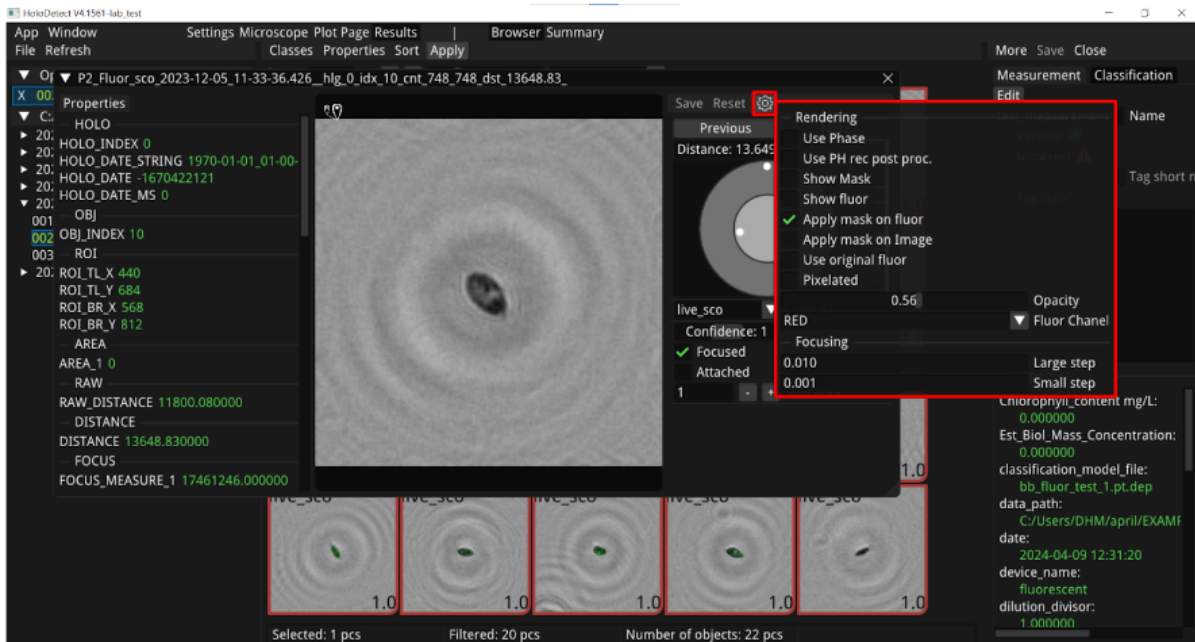
When you open a measurement, you will first see the objects found in the measured sample, sorted into classes. Select an object and right-click on it: a list will pop up offering several options. You can open the selected image (Open Editor), or you have the option to reclassify the selected object(s) by selecting the Annotate selected option. You can modify the selection, select all objects, copy/paste selected images (if you want to copy images from one measurement to another) and delete them.



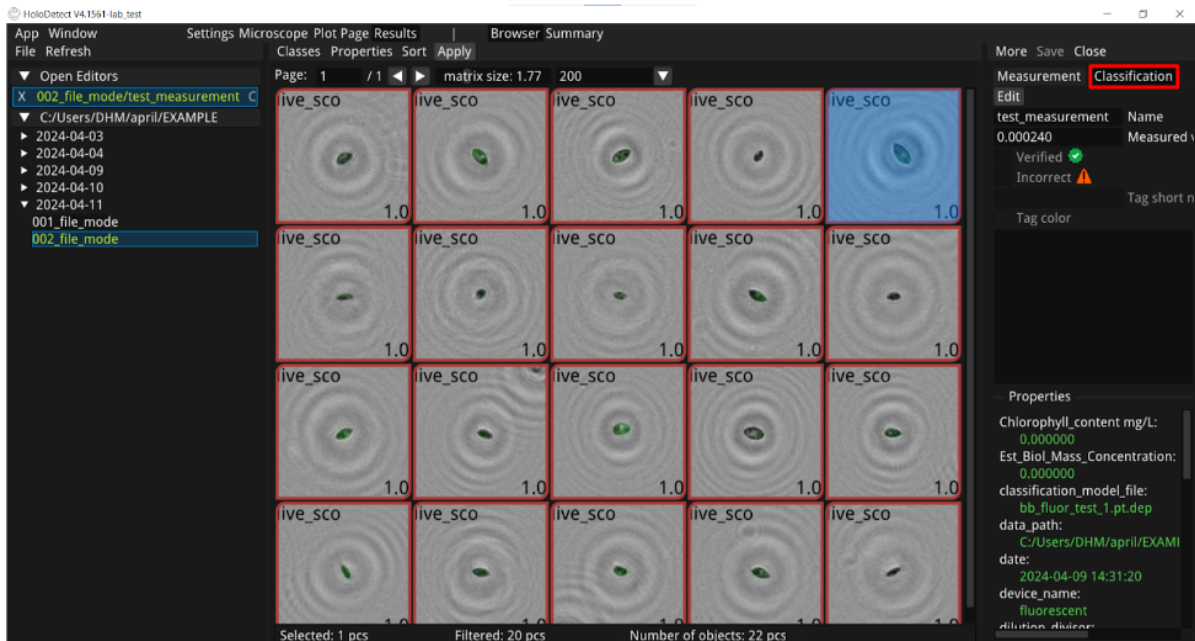
Select any image and press the Open in Editor option to open it. Here you can adjust the focus of the image using the sliders on the right, change the Class ID of the image, and adjust the number of cells in the image (Subcount) for a more accurate concentration count. You can select whether the opened object is in focus (Focused) or appears to be attached to the surface (Attached - in this case it is not included in the concentration count to avoid false results). Both Focus and Attached are parameters that can then be used to filter the objects you want to display later. To switch between images, use the Previous and Next buttons.

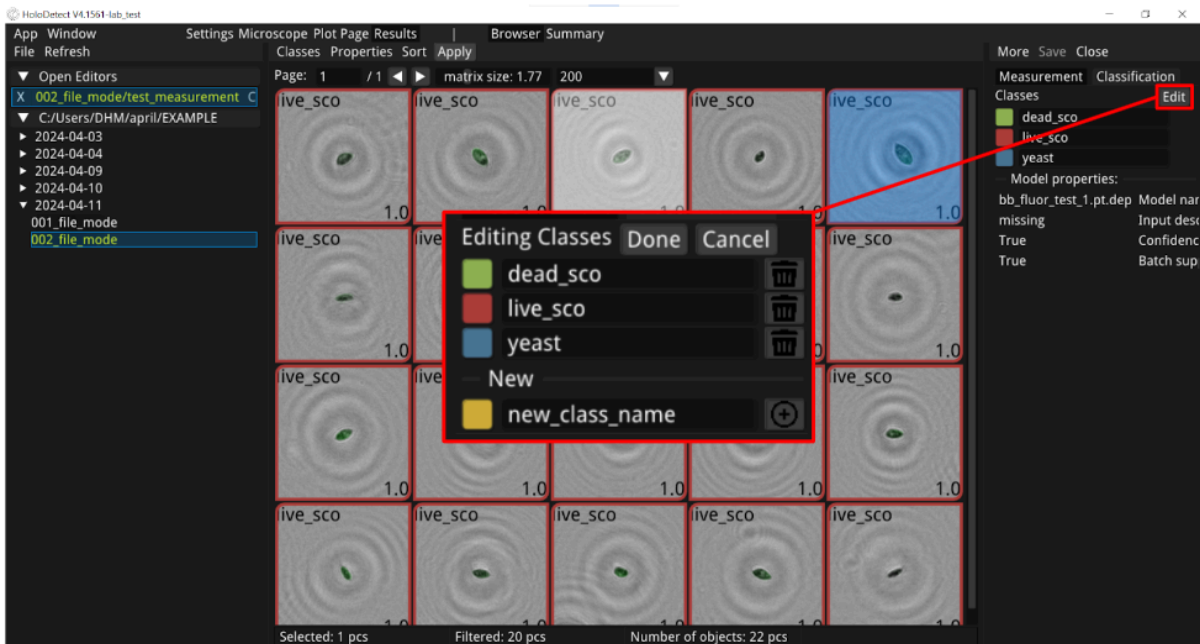


By clicking on the gear icon, you can choose which image to display:

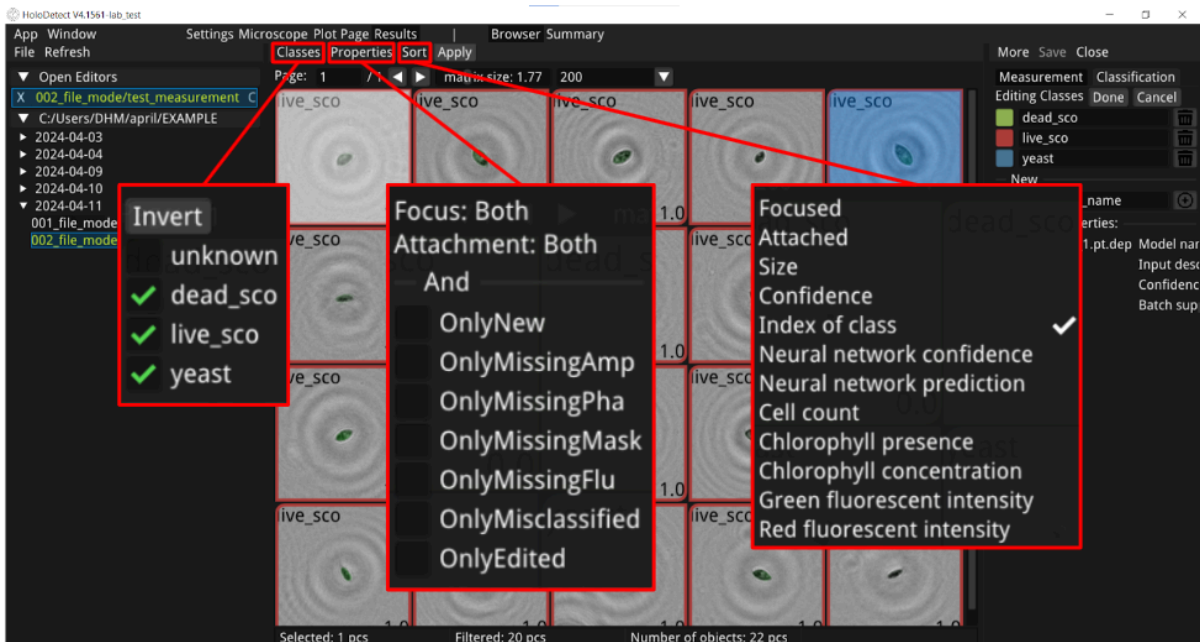


Clicking on the Classification tab on the right will display the currently existing classes and their corresponding colors. It is possible to create a new class and delete an existing class by first pressing the Edit button and then pressing the + or - icons. If you want to rename an existing class, click on the Edit button, then click on the name of the class you want to change and enter the new name. Once you have finished making changes, press Done. To save all changes, press the Save button.



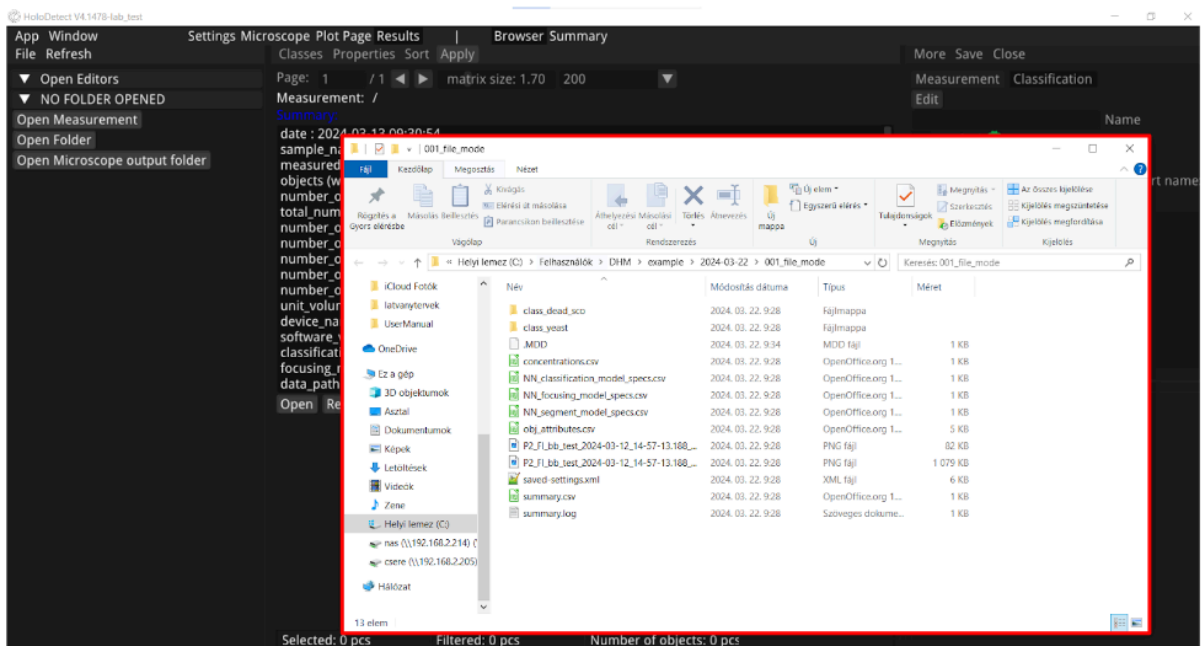
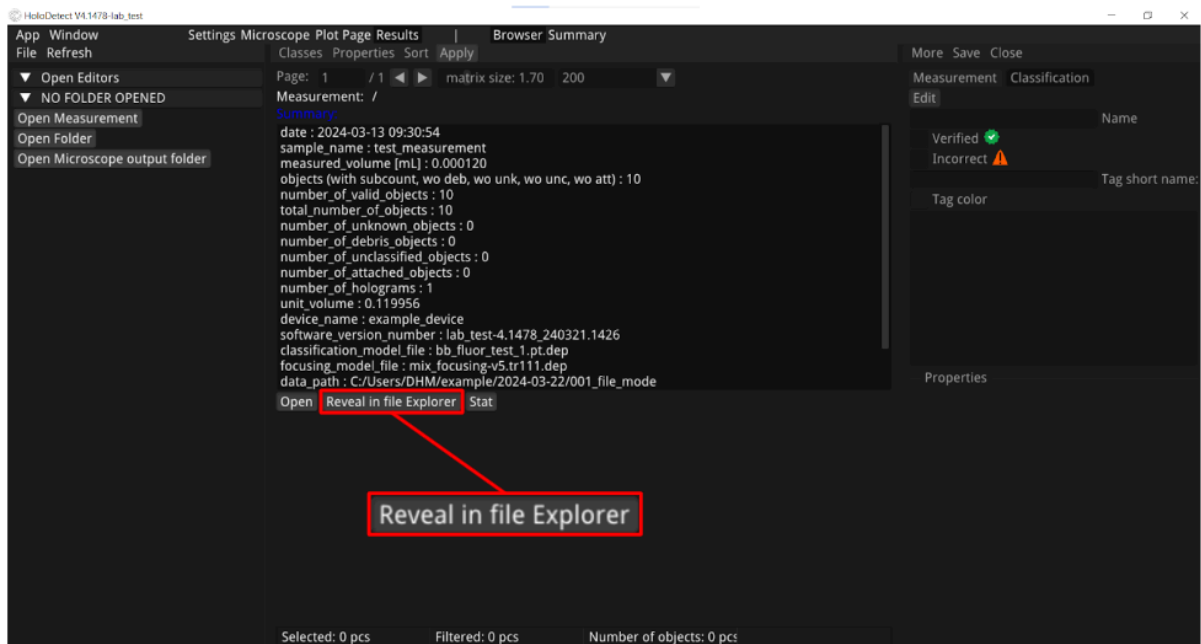


There are several ways of displaying images: the Classes button lets you choose which objects belonging to which classes to display, the Properties button lets you display objects based on the properties of the images, e.g. whether they are missing a picture (e.g. the phase, the fluorescence image, the mask), but also to display only edited images (e.g. with changed class or focus). The Sort button allows you to select the attribute according to which you want to sort the images.

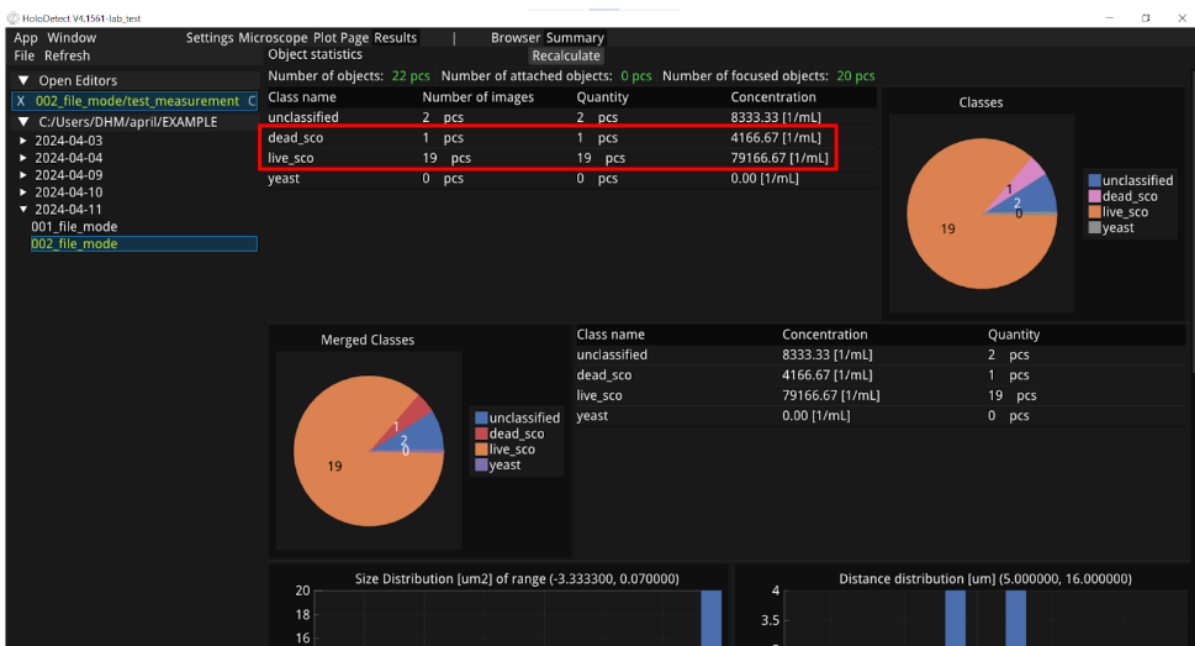
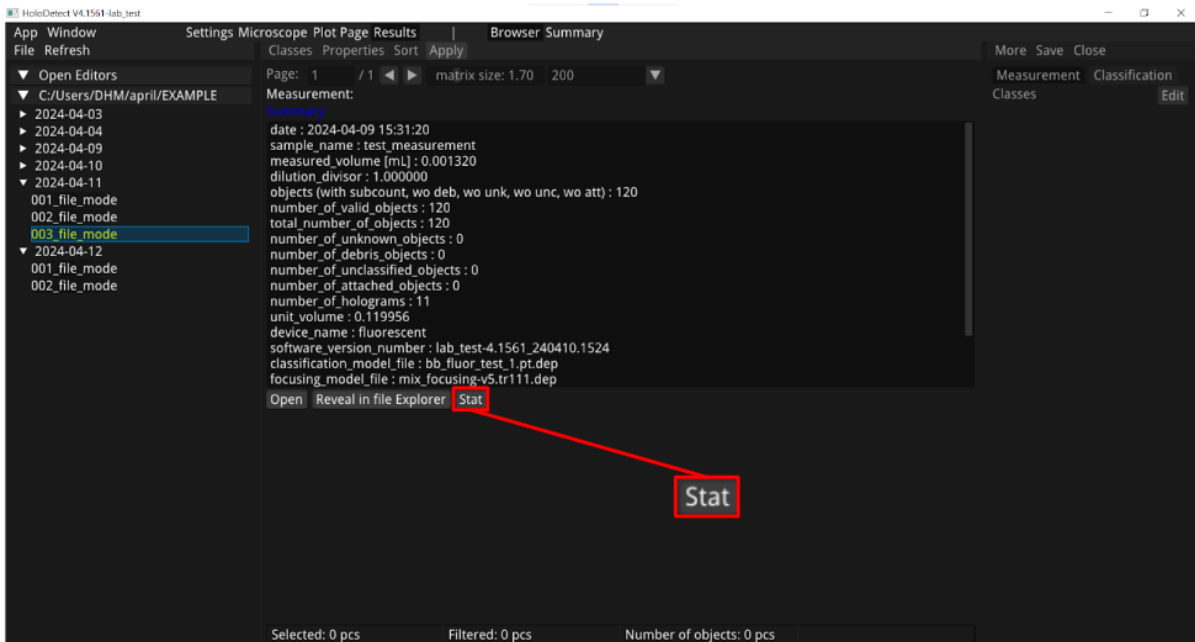


Before you open a measurement, you can open it in File Explorer by pressing the Reveal in File Explorer button. Here you can view the holograms saved during the measurement (if

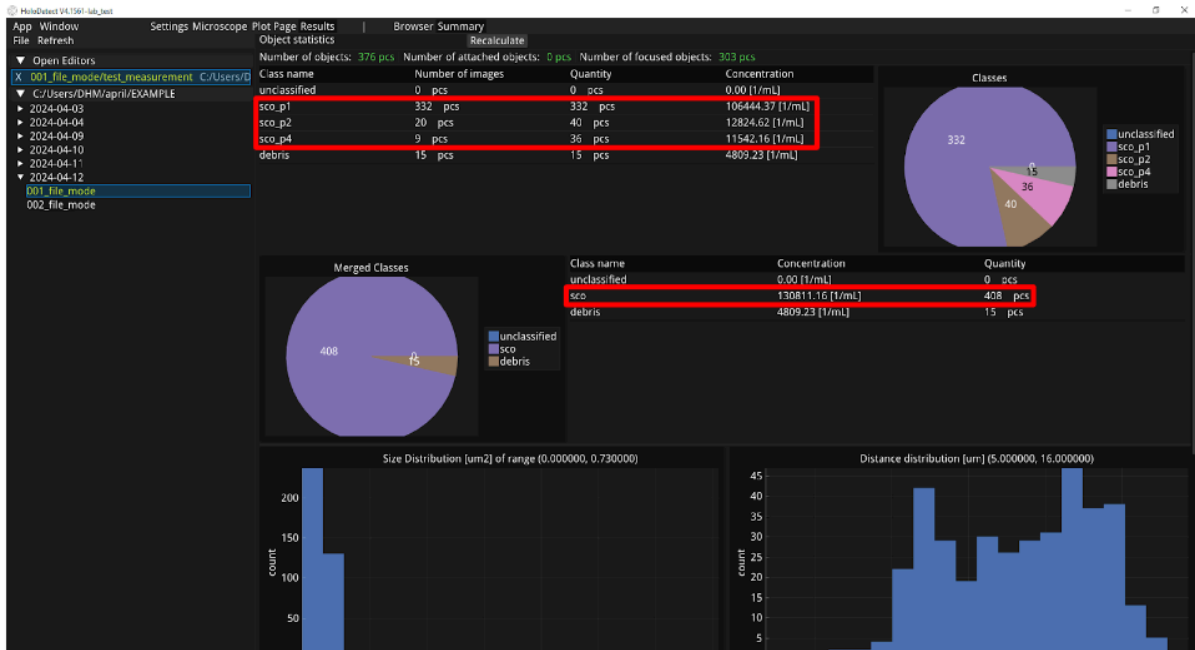
saving is enabled), the folders containing the objects and the files describing the measurement results in the usual folder system.



If you only want to open statistics for a specific measurement, you can do so by clicking on the Stat button when you select a measurement from the list on the left. The images are then not displayed, only the pie charts and tables containing the data.



The first table will list each class separately, but if you have used the naming convention mentioned earlier (classname_p1, classname_p2, where the classname must be the same in all cases, followed by an underscore, then a letter p and a number), the second table will contain the combined values of these classes.



Miscellaneous

For detailed *terms and condition of sale* of the Holodetect devices please refer to:

https://www.holodetect.com/assets/files/Holodetect_TERMS_AND_CONDITIONS_OF_SALE.pdf

Appendix A

Current and Future Functions of the Holodetect Devices

Holodetect sensors and devices developed by Holodetect Instruments Ltd. utilize Digital Holographic Microscopy (DHM) and artificial intelligence to automatically analyze volumetric liquid samples to detect, classify, and count objects, cells, and microorganisms in the 3-400 micron range.

Holodetect DHMs are well suited for inline, automated monitoring of the following parameters:

- Cell count by class. In monocultures, Holodetect can distinguish algae cells from other objects in the sample (debris, small objects etc.). In multi-species cultures it provides cell count by species. It also gives an accurate estimate of cell count in cell conglomerates.
- Detailed cell morphology (cell size, shape parameters)
- Measure of autofluorescence and estimate chlorophyll (or other auto fluorescent molecules) content.
- Living and dead cell counting with labeling.

Holodetect devices connect to the sampling gate of a fermentor/bioreactor through a Holodetect sampler, which has the following functions (expected to be available in Q3 2024):

- Takes a predefined volume of sample from the reactor through the sampling gate.
- If necessary, it dilutes the sample to levels suitable for the holographic microscope.
- Homogenizes the sample.
- Feeds the sample to the microscope in a controlled way.
- Measures optical parameters of the sample (OD, turbidity etc) - a feature under development
- Staining samples for labeled measurements (e.g living/dead cells, bacteria) is also a feature under development.
- It also serves as a hub to connect a single DHM to multiple reactors for cost efficiency.

When deploying multiple Holodetect devices in a farm, a Holodetect server will be set up that collects measurement data from the individual devices that can be further processed, queried and reported. Real time measurement data provide essential feedback to the control loop of the fermentation reactors to automate the culturing process.

