Holodetect User Manual

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Document history

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Introduction

Detecting microparticles, microorganisms, and 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 is 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 to use 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 and microparticles. It can flexibly integrate with a wide range of industrial equipment. The developed instruments feature measurement cuvettes with depths ranging from 100 to 10,000 micrometres to accommodate different magnification needs.

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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; 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, HiRes + MultiFluor), 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 LVol) 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 LVol 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-view of the two microscopes needs 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 that might be required at 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 the excitation of Chlorophyll A molecules with a wavelength of 450 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 LV	Holodetect LV+Fluor	Holodetect HiRes	Holodetect HiRes+Fluor	Holodetect HiRes+Fluor3
Dimensions (W x L x H)	30 x 48 x 35 cm	30 x 48 x 35 cm	30 x 48 x 35 cm	30 x 48 x 35 cm	30 x 48 x 35 cm
Weight	8 kg	8 kg	7.5 kg	8 kg	8 kg
Detectable object size range	3-300 µm	3-300 µm	3 - 75 µm	3-75 µm	3-75 µm
Holographic Resolution	1.1 µm	1.1 µm	600 nm	600 nm	600 nm
Measured volume	0.1-6 ml/min	0.1-6 ml/min	3,6-18 µl/min	3,6-18 µl/min	3,6-18 µl/min
Flow-through cuvette depth	200 µm - 10 mm	200 µm - 10 mm	200 µm	200 µm	200 µm
Processing hardware configuration	intel i3 14100 + RTX 4060	intel i3 14100 + RTX 4060	intel i3 14100 + RTX 4060	intel i3 14100 + RTX 4060	intel i3 14100 + RTX 4060
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	-	3.45 µm	-	3.45 µm	3.45 µm
Excitation wavelength	-	Customizable (450nm, 488nm, 532nm)	-	Customizable (405, 450nm, 488nm, 532nm)	405nm, 450nm, 488/532nm
Emission highpass filter	-	550nm	-	550nm	550nm

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

AI-Trainer 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.

AI-Trainer 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

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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 (fine-tune, or 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 AI-Trainer module can be found in the *Using the AI-Trainer Learning Module* section.

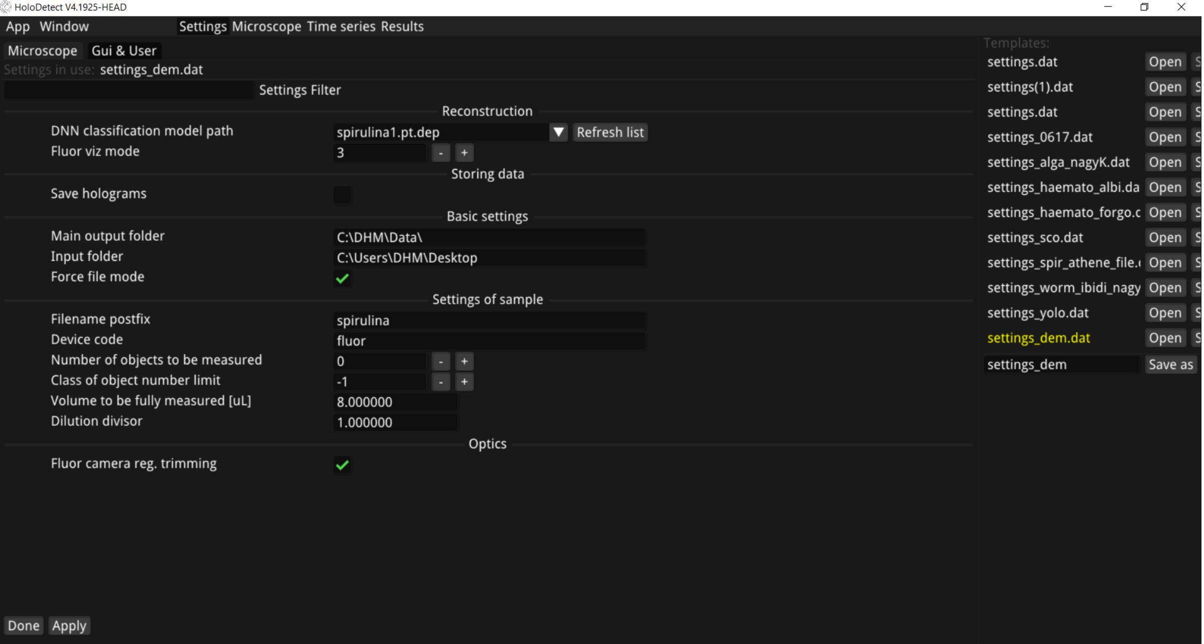
Sample input

Insert the input tube into your sample container (e.g., Falcon tube or flask) and the output tube into a waste container. Make sure that your sample is homogeneous and does not settle during the measurement.

Calibration session

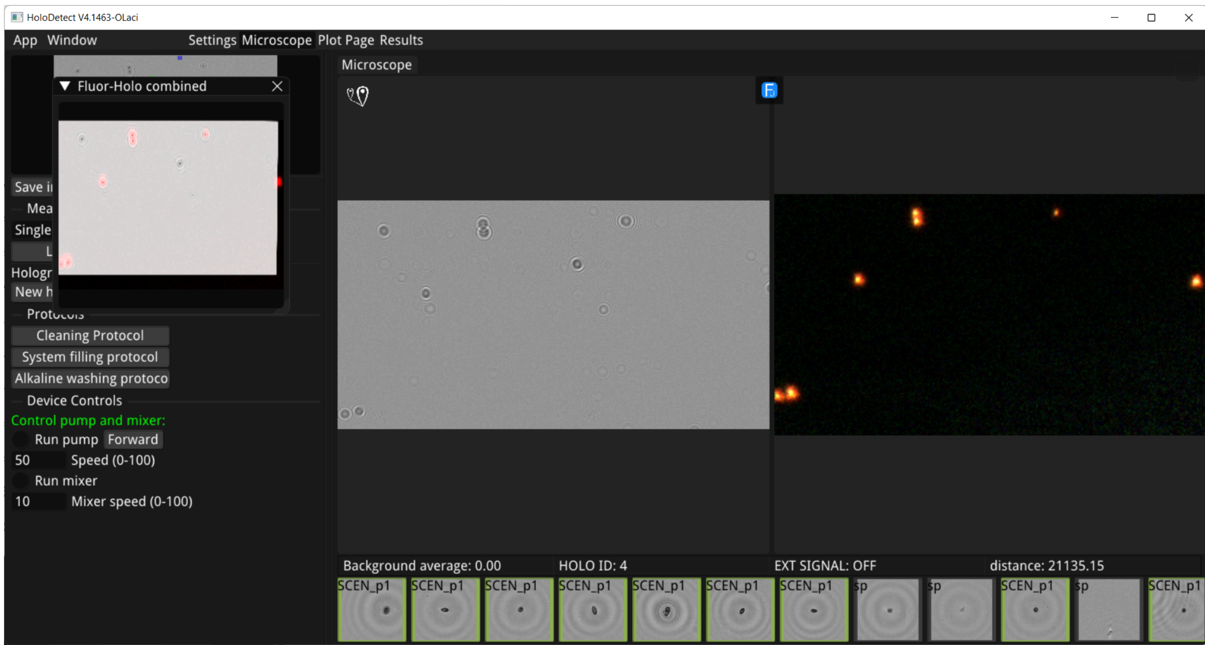
As there are two different cameras, their actual view of the sample has to be matched. This is done by calibration. The magnification difference and the rotation of the two views are set and optimized during the device construction using calibration tools that can be achieved in the advanced mode only. 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 flow-through 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).

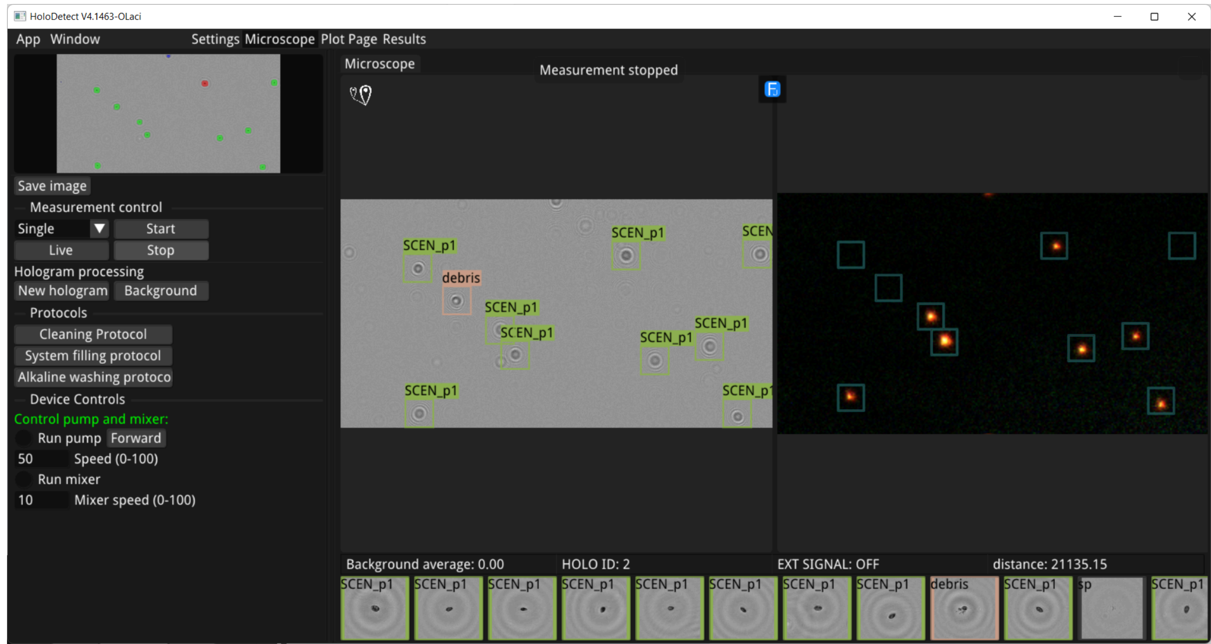


Then start a measurement. At the end of the measurement, the offset will be corrected.

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.



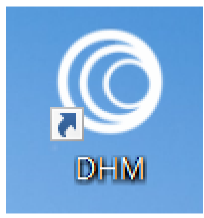
Please, save the actual settings to ensure the calibration for consecutive measurements. If the calibration is apparently not satisfactory, please run the calibration process again (with the previously saved settings - or - if the mismatch is small - with the current settings).



The Software of the DHM

Starting the DHM application

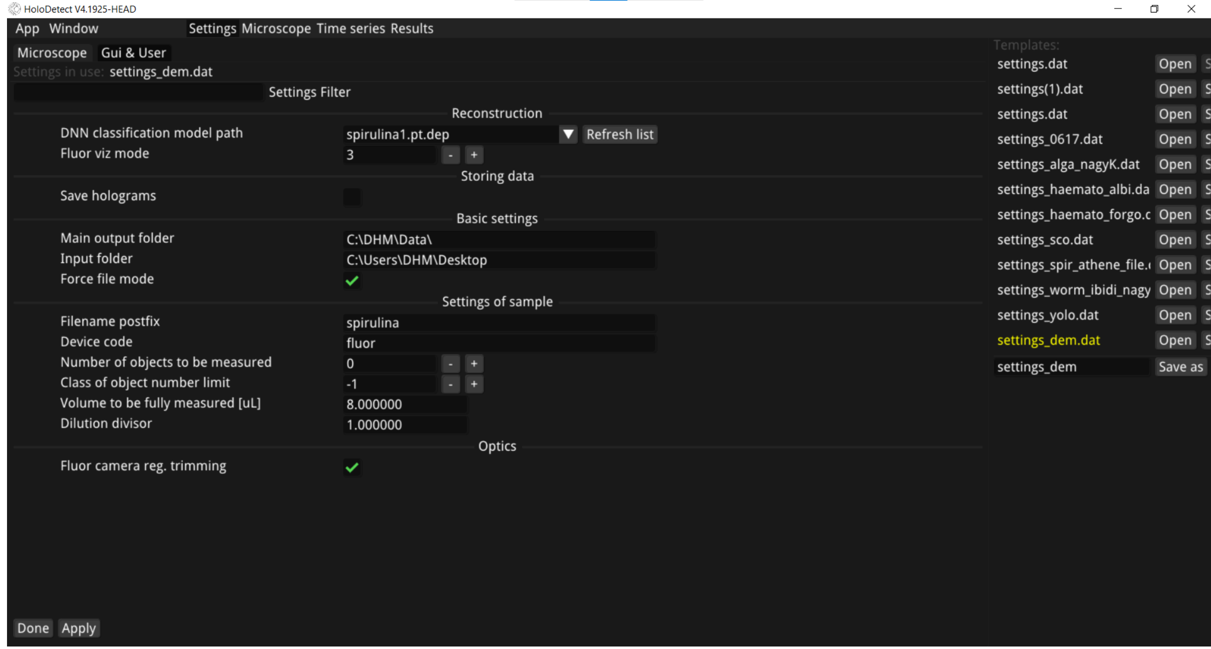
Click on the icon to start the Holodetect's Digital Holographic Microscope (DHM) application.



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 file*, 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

- ***DNN classification model path***: a list from which the classification neural network model we want to use can be selected.
- ***Fluor viz mode:*** which fluor image should be displayed during the measurement:
  - 0 - merged that combines all wavelength
  - 1 - the first one
  - 2 - the second one

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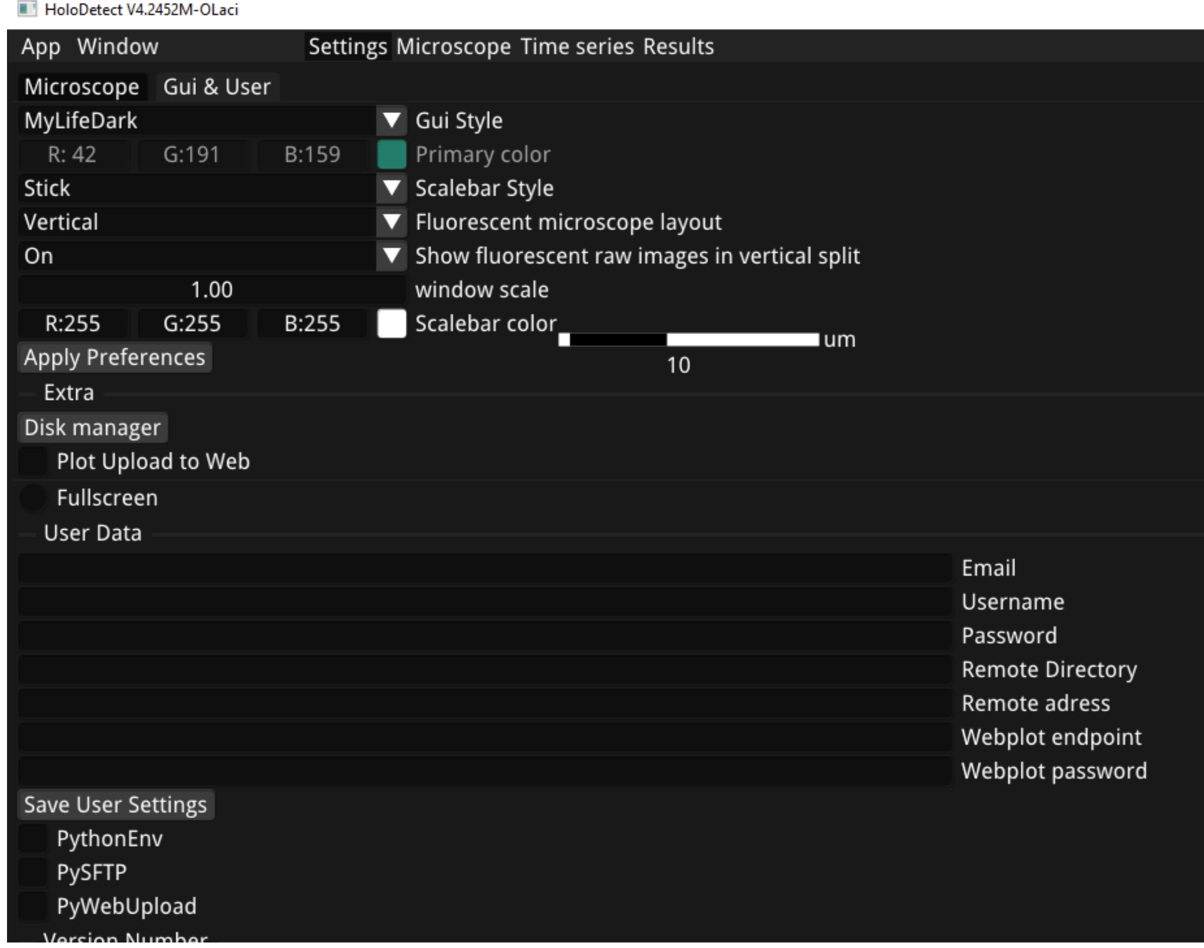
- 3 - the third one

  The Fluor viz mode has to be set to 1, when all the fluorescent measurements are to be shown. To achieve this, the user has to set the Fluorescent microscope layout to vertical in the GUI & User panel. The proper set can be seen in the following figure.



- ***Save holograms***: Save the recorded, background-compensated, full-sized holograms as well. This option serves development purposes as it stores most of the raw information and consumes big disk space. It provides the possibility to re-evaluate measurements with different settings later. Hence, it is recommended to record rare or otherwise precious samples with this option set to yes.
- ***Main output folder***: All the measurements are saved under this path in a 2-level subfolder structure: “day of measurement” / “index mnemonic of measurement” (E.g.: 2024-12-01/001).

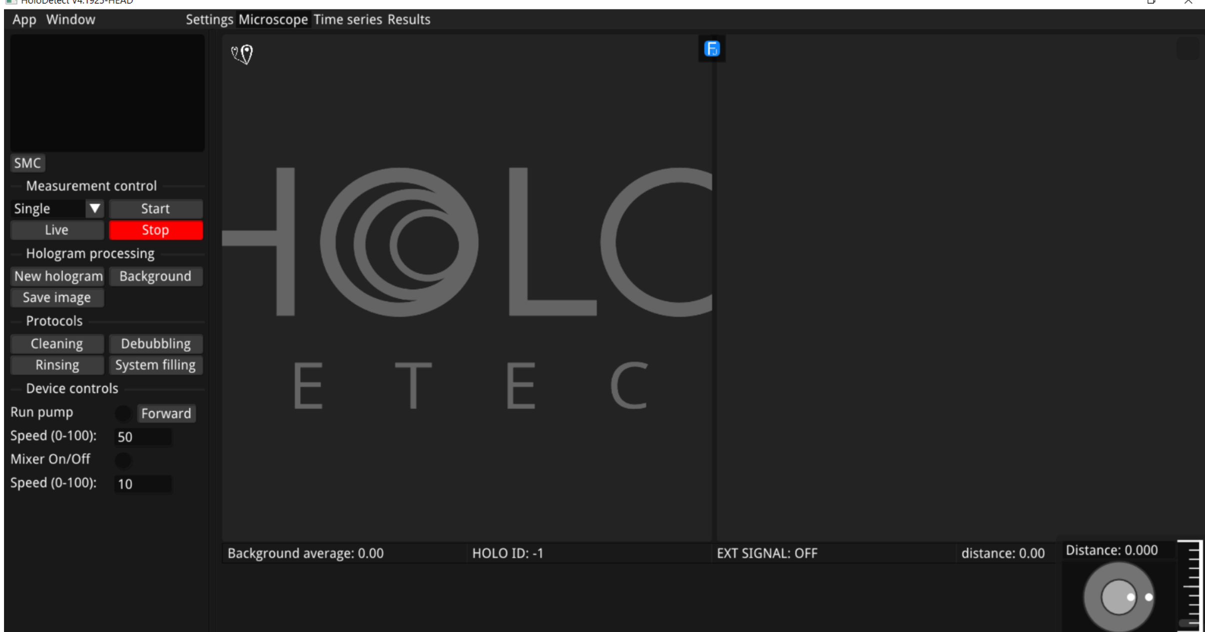
- ***Input folder***: The folder of previously recorded holograms to be processed. If there is no camera available, the program tries to load holograms from this folder. Use '\' as a directory separator. E.g.: C:\Measurements\2021-06-12\001.
- ***Force file mode***: If this option is true the program works from this folder instead of using the camera.
- ***Filename postfix***: Optional postfix for the name of the images. It should not contain special character combinations like: "fluor", "flu", "holo", parentheses, and blank space characters.
- ***Device code***: ID of the device.
- ***Number of objects to be measured:*** when the number of found objects reaches this value, the measurement stops. This stopping condition overwrites the given time or volume criteria if the set value is greater than zero.
- ***Class of object number limit***: if this value is greater than -1, the number of objects to be measured is applied with this class ID, that is, the program considers only objects of this class when it checks the stopping condition. It takes effect only if the object number-based stopping constraint is given. The ID of the classes starts from 0 (unclassified -1) and follows the order seen e.g., on the local annotation menu of the Results page.
- ***Volume to be fully measured***: the Holodetect HiRes and HiRes+Fluor measure 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. The measurement time of a hologram is a few seconds, depending on the number of objects in 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, because the microscope's field of view is much smaller than the width of the cuvette. After the measurement of this volume, the system begins a new measurement.
- ***Dilution divisor:*** 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..
- ***Fluor camera reg. trimming:*** The program performs an automatic calibration of the fluorescent camera to the holographic one based on the first ten image pairs. To this end, a previous rough manual calibration is needed to have mainly overlapping wavelengths

regions on the two cameras of a similar size. It switches this option off right after the operation finishes.

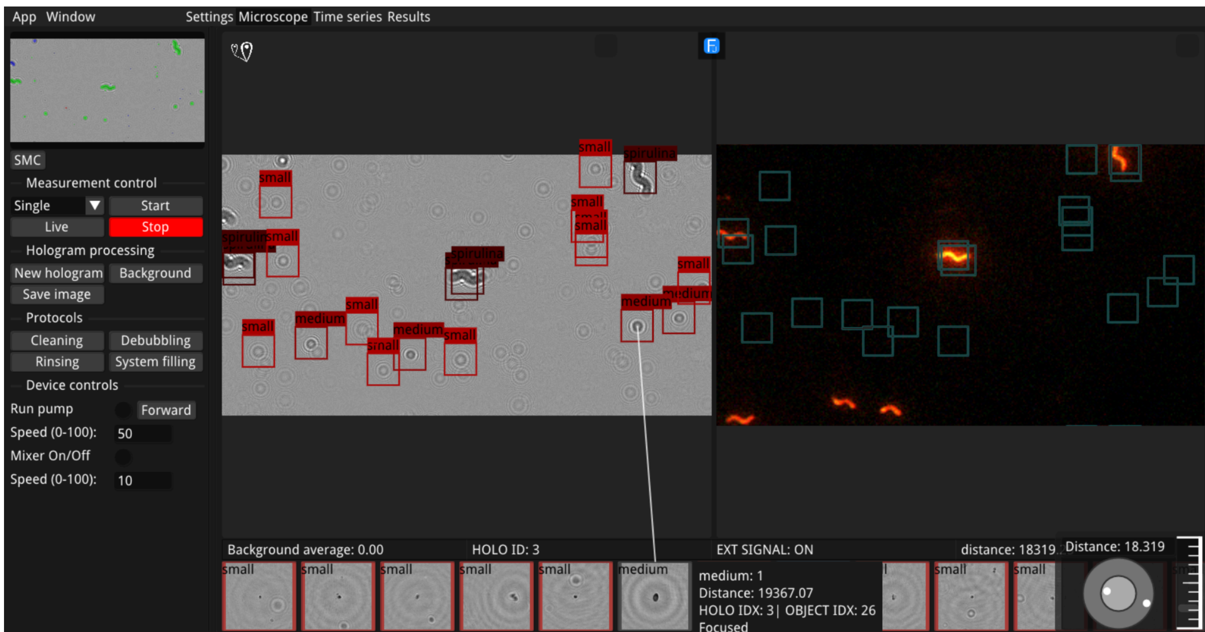
- On the right, different letters indicate different options:
    - ***Use:*** the selected Settings file will be loaded by the system.
    - ***S:*** The selected file will be saved.
    - ***R:*** Rename the selected file.
    - ***D:*** duplicate the selected file
    - ***X:*** delete the selected file.
- ***Save as:*** the loaded Settings file can be saved with a new name.
- **Apply button:** Use the previously set parameters.
- **Done button:** Apply the parameters previously set and move the focus from the Setup window to the Microscope window, where the measurement can be started. If you do not press either Apply or Done, the settings will not be saved!

Microscope Page

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



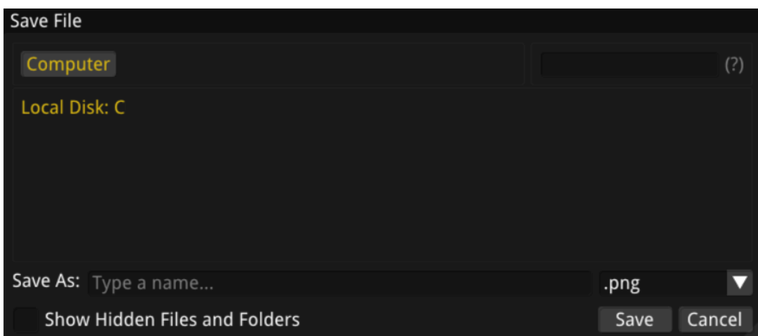
By clicking the central Fluor icon (capital F) the fluor display will also appear next to the hologram. The centers of the measured fluorescent and hologram objects should be aligned.



This can be achieved using the built-in auto-calibration tool (See Calibration session!). During the measurement, the objects are displayed at 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, the focus of the whole hologram is moved to the reconstruction distance of this object, and its position is shown by a connecting line segment.

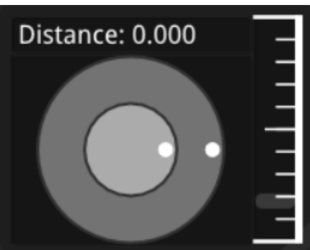
All measuring and microscope handling tasks can be controlled by buttons that are located on the left panel of the window. These buttons are as follows:

- *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

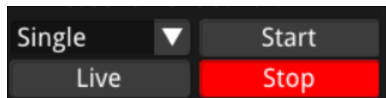
There is a Focus Knob in a standalone window, which works similarly to that of conventional microscopes and makes the manual focusing of the holograms possible. It contains fine adjustment and coarse adjustment knobs, which enable fast and accurate manual focusing. Besides these ones, there is a focusing slider when large-scale focus adjustment is required.



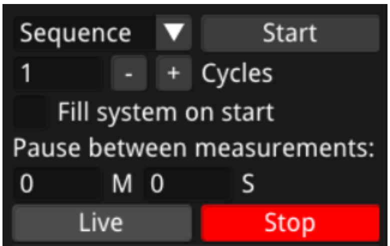
The actual position and parameters of this window can be chosen by pushing the right mouse button and selecting the proper element from the list.

Measurement Control

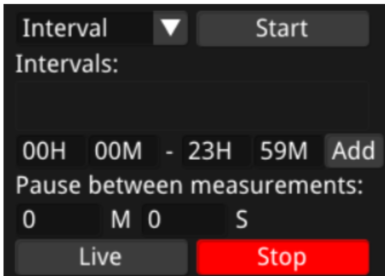
- In this panel, you can set the actual type of measurement:
  Single, Sequence, Interval, Continuous.
- In *Sequence*, *Interval,* and *Continuous* modes, it is possible to set a parameter, *Pause between measurements*: this determines the time interval between each measurement, given in minutes (*M*) 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.

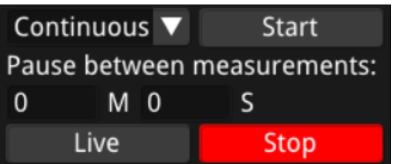
  - *Sequence:* In this case, the DHM takes a series of measurements from the given sample. The *Cycles* value should be set according to the number of consecutive measurements. Then, by selecting the *Fill system on start*, a sufficient amount of sample can be loaded into the system (if a sample will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

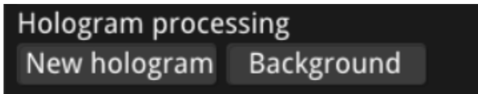


- *Continuous* - In this mode, the measurement will not stop automatically unless the *Stop* button is pressed.



- All types of measurements can be started by pressing the *Start* button, but only the *Continuous* measurement requires 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 to live mode. In this mode, the main window displays the measured holograms in 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 with the calibration and test if the DHM is 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 also be supervised here. 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

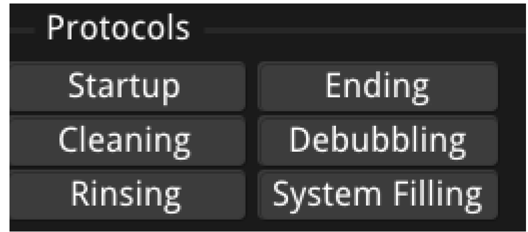
- *Startup Protocol*
- *Ending Protocol*
- *Cleaning Protocol*

  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.
- *Debubbling Protocol*

  Removes bubbles from the system.
- *Rinsing Protocol*

  Between 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.
- *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.

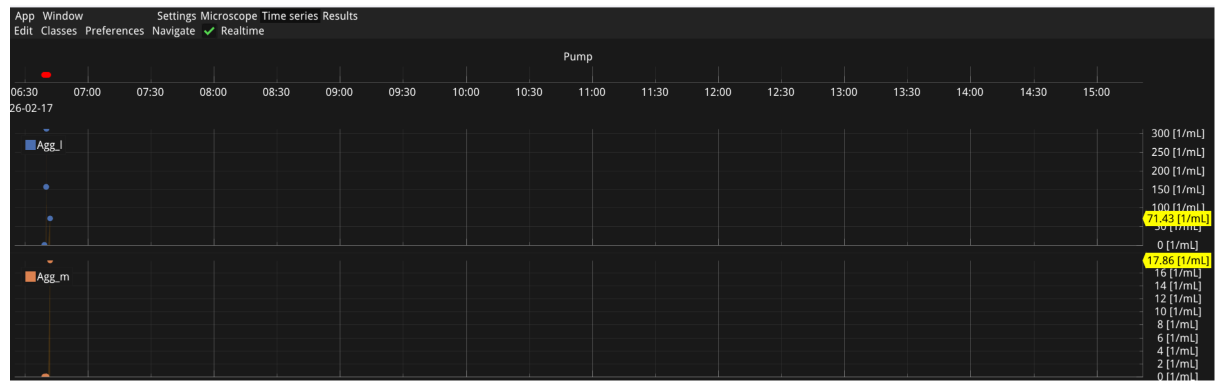


Device Controls

- *Run pump*: The pump speed can be adjusted on a scale from 0 to 100, with a default value of 50.

Time series

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 the time difference is greater than specified). A breakpoint is inserted between non-connected measurements.

You can select the 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.

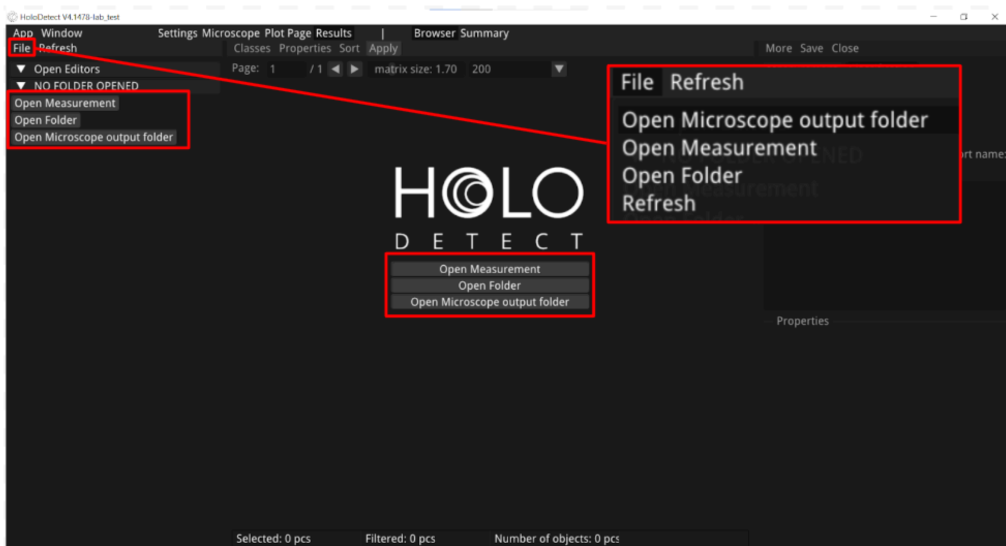
“Realtime” option will keep the time axis on the current time.

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 is 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

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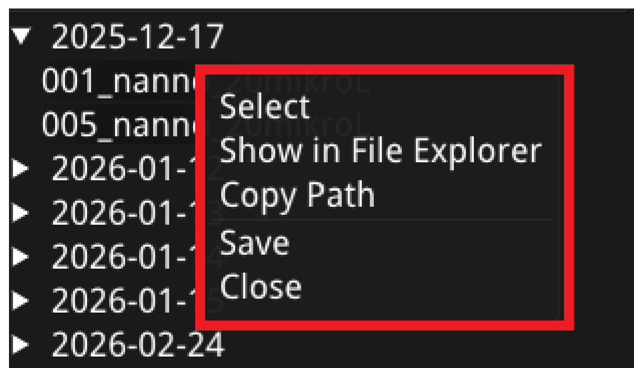
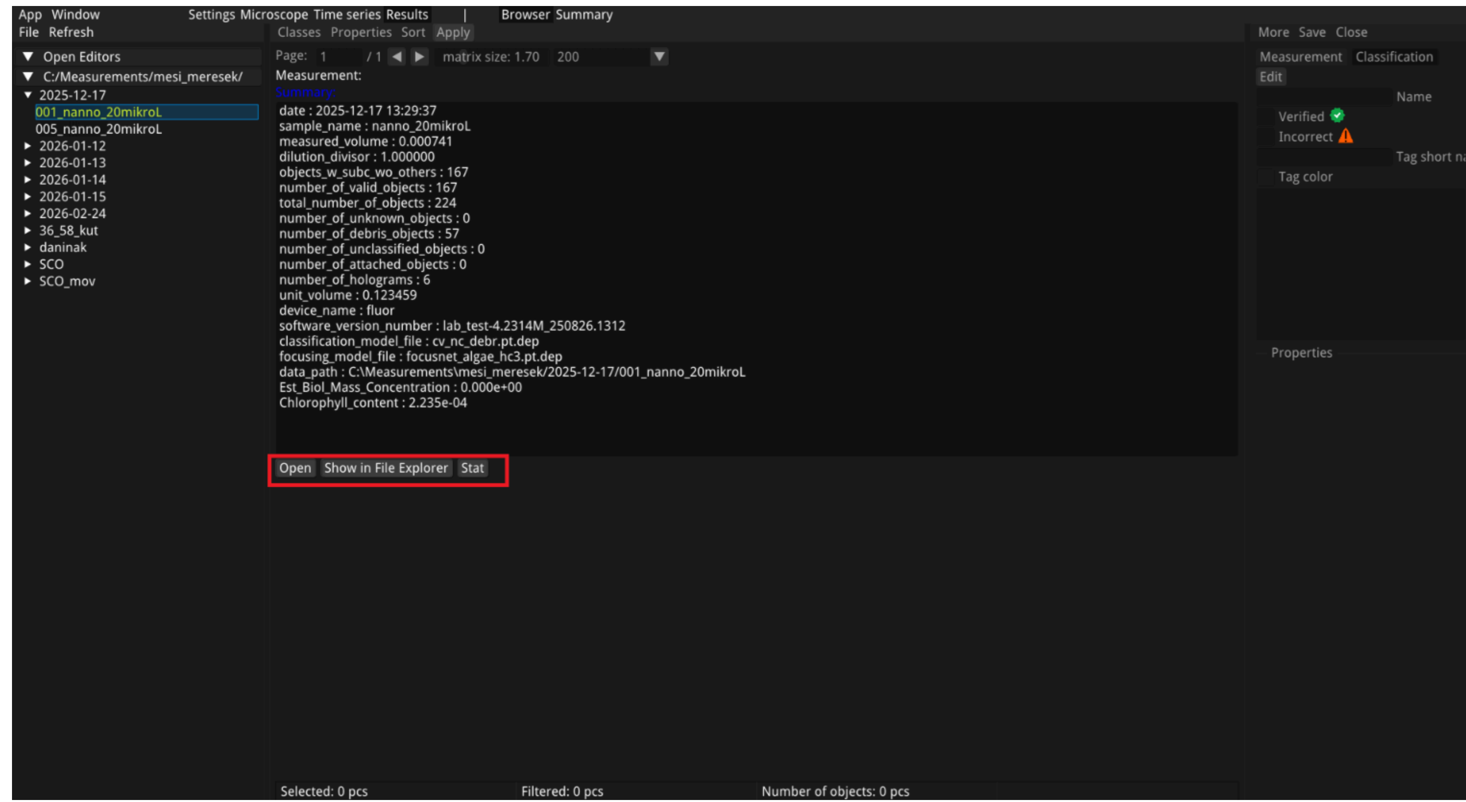
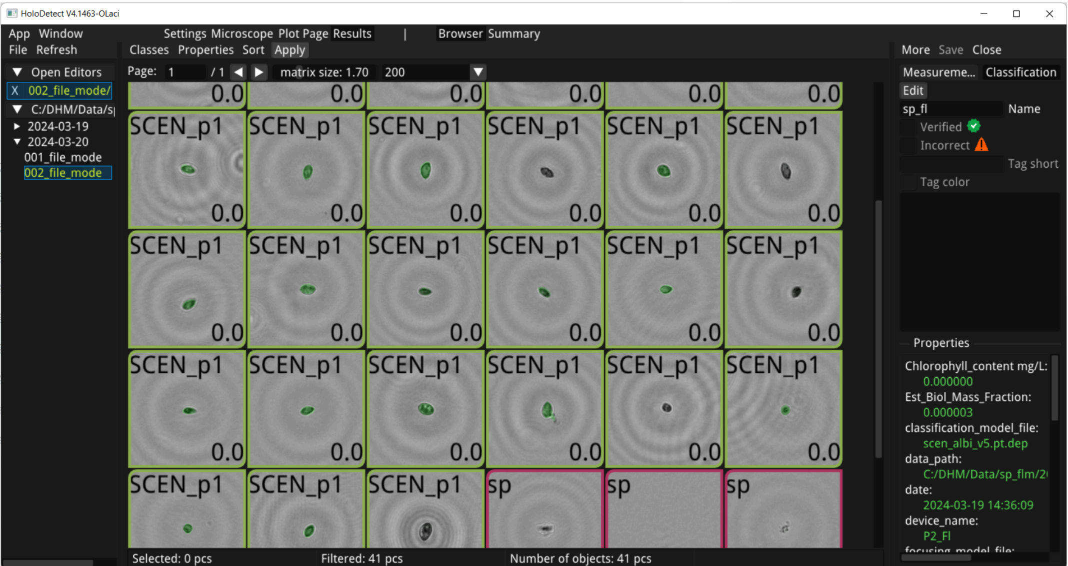
move the mouse over them, you can see their paths, and right-clicking on them will bring up several options: *Select* to select and open the measurement folders, *Show in File Explorer* to open the measurement folder outside the program, *Copy Path* to copy the path, Save to save changes, or *Close*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



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

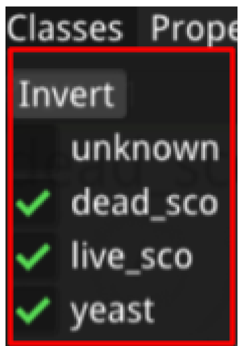


- There are three buttons to choose from: open the measurement with the *Open* button, open the measurement folder in Windows Explorer with the *Show in File Explorer* button, and open the measurement statistics by clicking on the *Stat* button.
- Select a measurement and open it with the *Open* button.

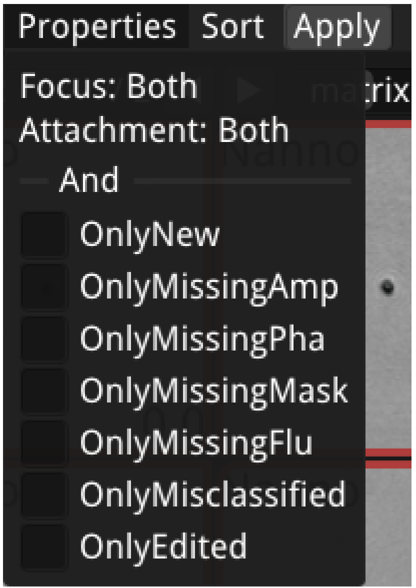


- The objects in the sample are displayed, sorted by their classes. At the top of the page, you can set the size of the matrix to be displayed by setting the Matrix size to (default value 1.70), or you can set the number of images to be displayed per page from 100 to 1000 (default value 200).

- 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)

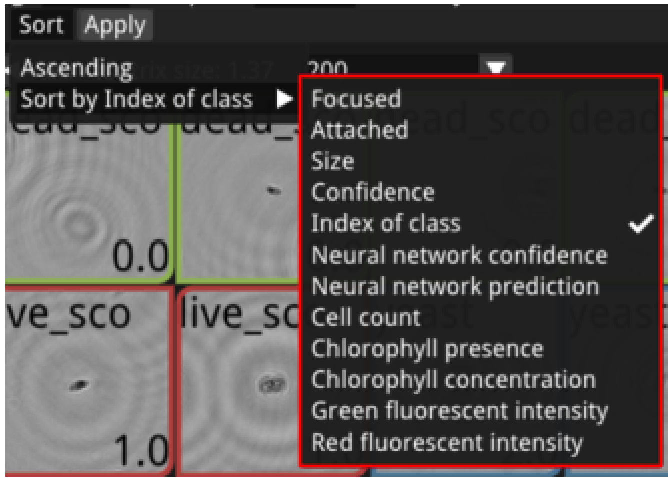


    - *Show only 'Focused'*: If you click, you can choose 3 options: Focused, unfocused, or both, depending on what you would like to see.
    - *Show only 'Attachment'*: displays objects stuck to the wall of the measuring cell. You have the same 3 options, like at the Focused.
    - *Show only 'OnlyNew':* If you copy an object and paste it into another measurement, you can select it. The program will remember this change after you save it, but not after closing the whole measurement.

- *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
- *Show only ‘Only misclassified’:* You reclassified these objects, which belonged in another class according to the Neural Network. It will remember to reclassify after saving and closing.
- *Show only ‘Only edited’: It’s like Only new;* however, you can close after saving your measurement, and you can select every copied image when you open it again.

Error is marked with an amorphous amoeba drawn.

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

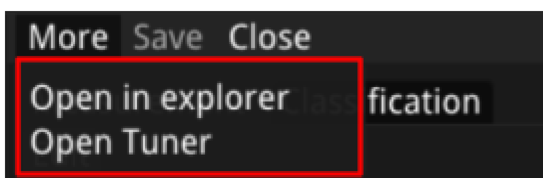


- *Focused*: Indicates whether the object is in focus or not.
- *Attached*: Indicates whether or not the object is attached to the wall of the measurement cell.
- *Size*: Specifies the estimated size of the object in pixels.
- *Confidence*: Indicates how confident the neural network is in the classification 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. Their numbers are based on their classes.
- *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. It 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.

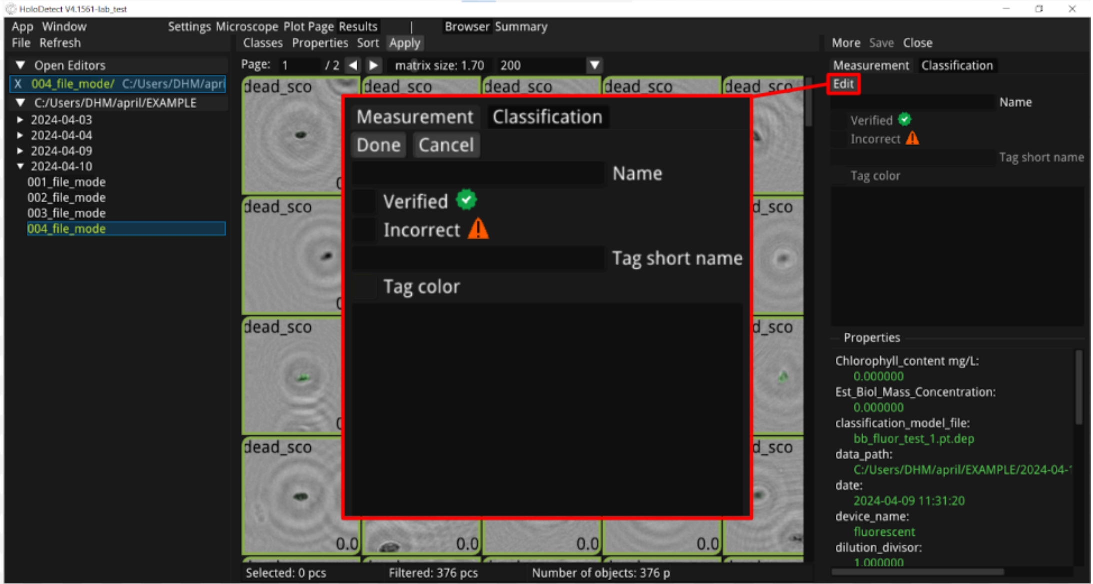
○ After selecting the filter parameter, click twice *Apply* to display the filtered images.

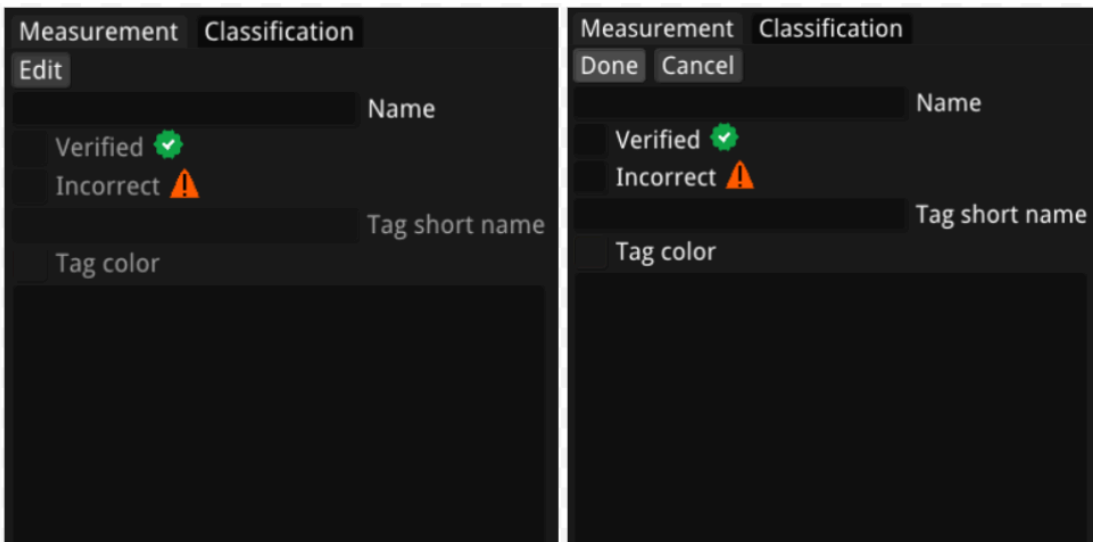
- Clicking on the *More* button in the top right corner allows you to open the measurement outside the program (*Open in Explorer*), or to open the Few-Shot Tuner window (*Open Tuner* - more on this later).

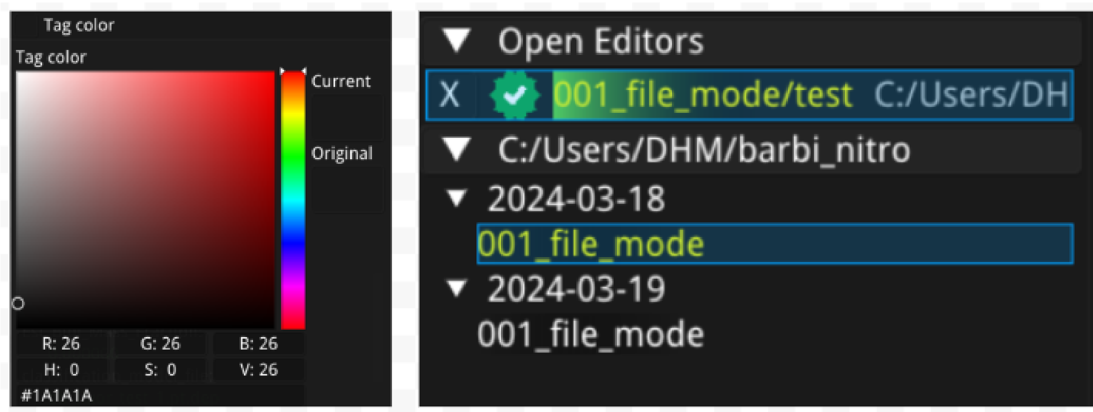


- By pressing *Save*, you can save the changes you have made to a measurement, and by pressing *Close*, you can close it (if another measurement is open, the view automatically switches to that one).
- If you change anything in your measurement, a small sign will appear right next to the name of the measurement, which warns you that you haven't saved your modifications yet.

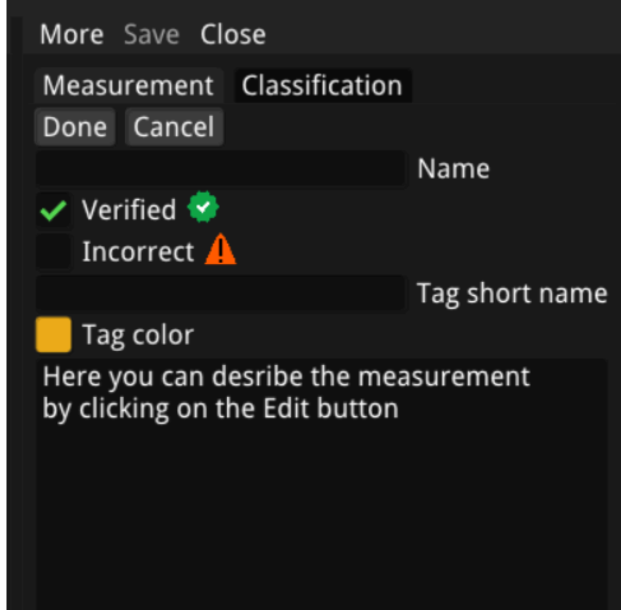
- By clicking on the *Edit* button under the *Measurement* tab, you can change the name of the measurement, you can tag it as *Verified* or *Incorrect* (these will appear as a green tick and an orange triangle next to the measurement name in the top left corner), and using *Tag short name* you can give 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).





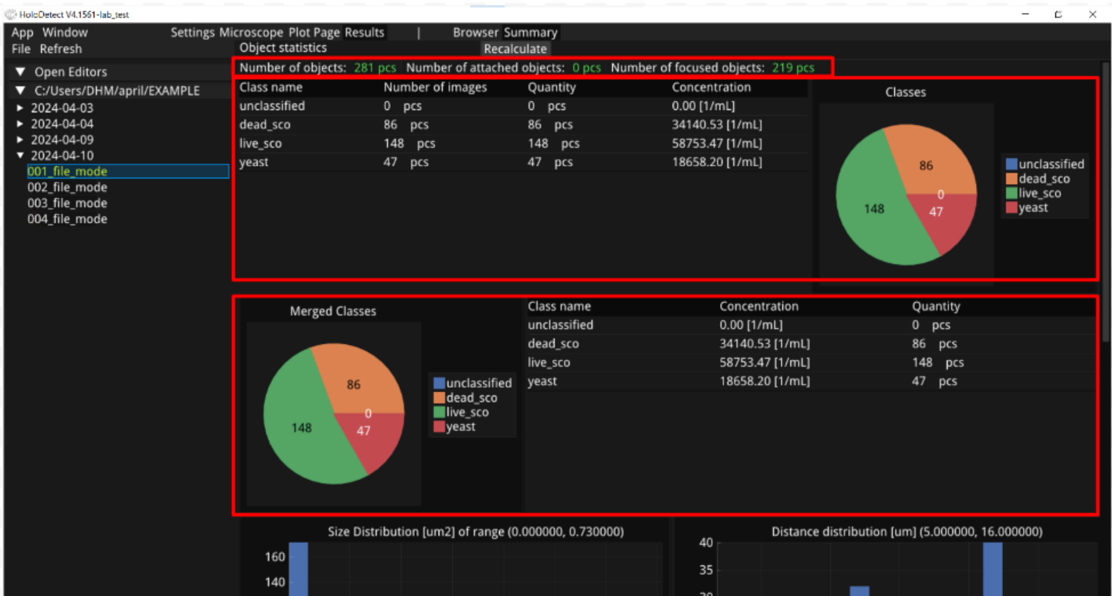


- There is also a text box where you can describe each measurement in more detail. Here you will also find some important information about the measurements (the same information is displayed when you select a measurement before opening it). To modify the *Name*, *Verified*, *Incorrect*, *Tag short name*, *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 desribe 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 sco_p1, sco_p2, and sco_p3. These will appear as scos in the second table and will contain all the cells from the listed classes. The

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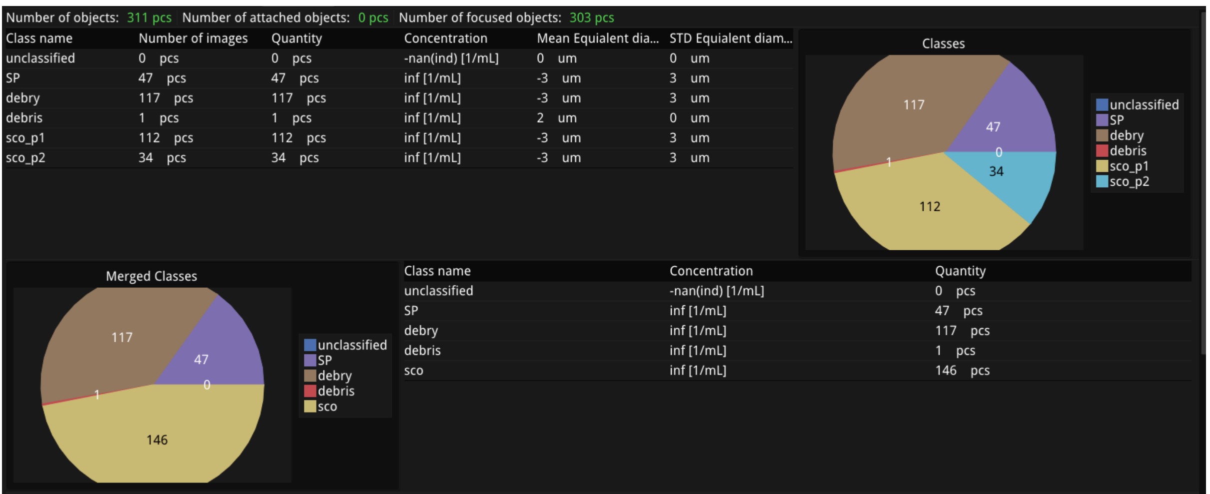
summation is based on the sub-counts, which can be modified if the neural network did not accurately estimate the cell count. (Such classes are best created when working with a sample that typically contains clumped cells. In this case, the class sco_p1 may contain single cells, sco_p2 may contain

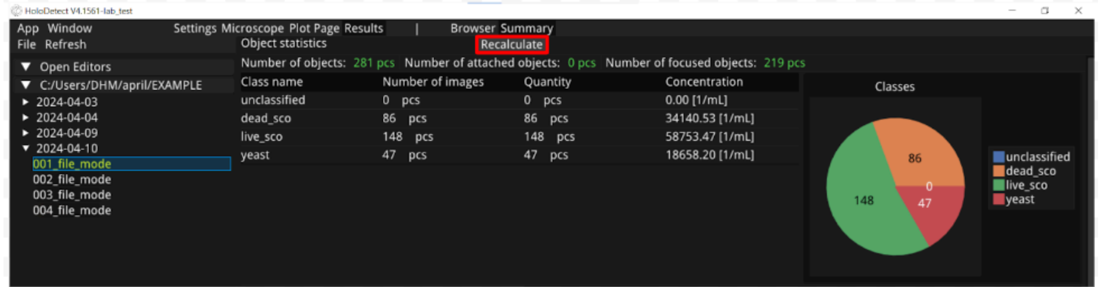


cells clustered by twos, and sco_p3 may contain cells clustered by threes.)

Eight bar charts. Description in *a step-by-step measurement demonstration.*

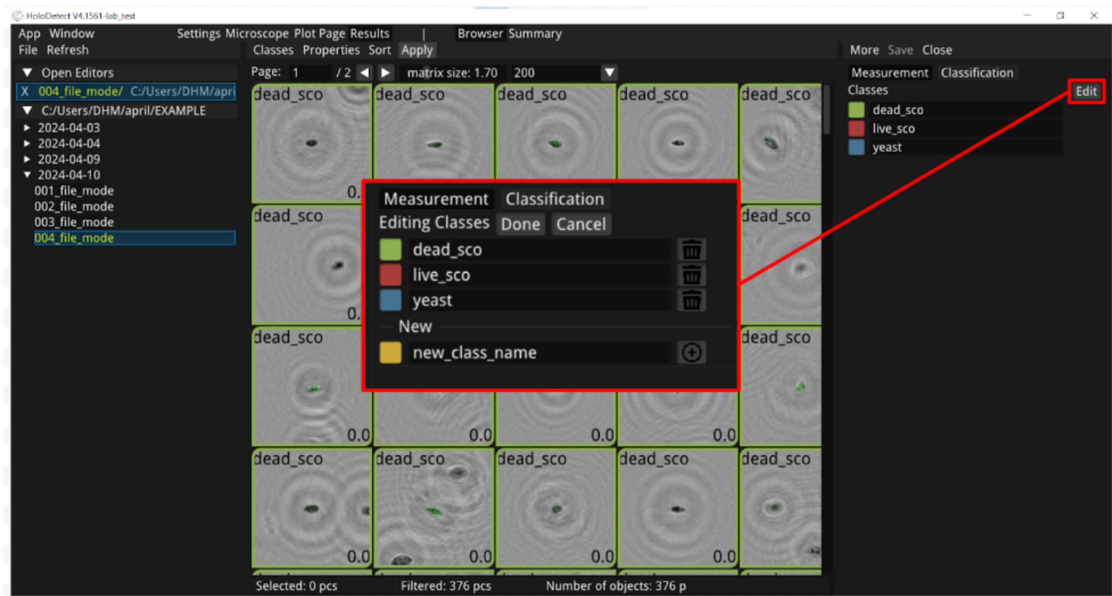
- If there has been a change in the Browser panel that has affected the classification, the Recalculate button can be used to update the statistics for the measurement.

HOLO
DETECT

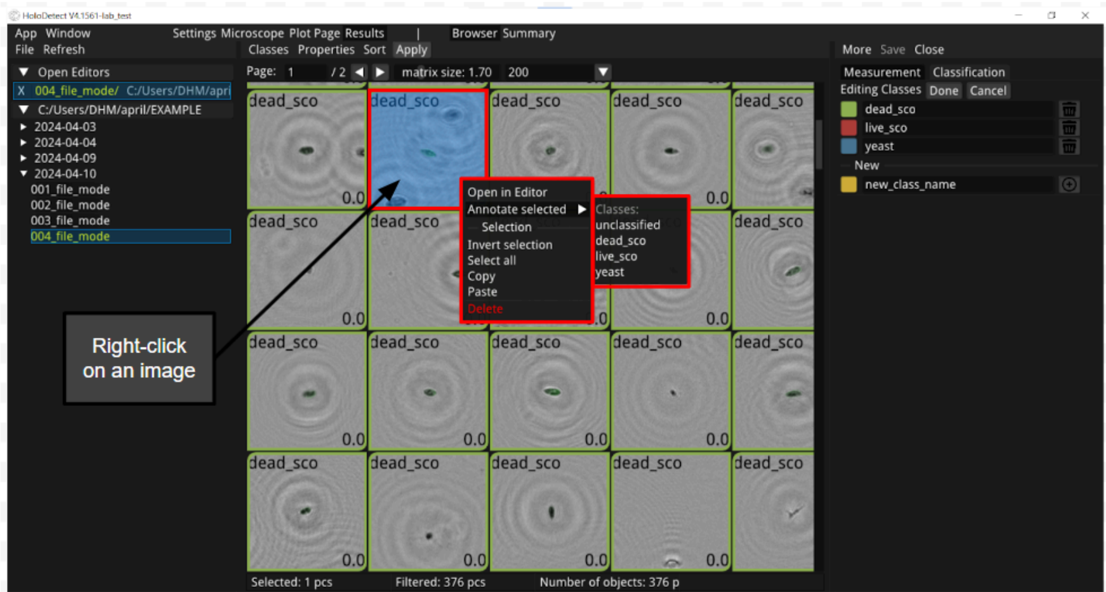

HoloDetect V4.1561-lab_test
App Window
File Refresh
Settings Microscope Plot Page Results
Browser Summary
Object statistics
Recalculate
Open Editors
C:/Users/DHM/april/EXAMPLE
2024-04-03
2024-04-04
2024-04-09
2024-04-10
001_file_mode
002_file_mode
003_file_mode
004_file_mode
Number of objects: 281 pcs Number of attached objects: 0 pcs Number of focused objects: 219 pcs
Class name Number of images Quantity Concentration
unclassified 0 pcs 0 pcs 0.00 [1/mL]
dead_sco 86 pcs 86 pcs 34140.53 [1/mL]
live_sco 148 pcs 148 pcs 58753.47 [1/mL]
yeast 47 pcs 47 pcs 18658.20 [1/mL]
Classes
86
0
148
47
unclassified
dead_sco
live_sco
yeast

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*.



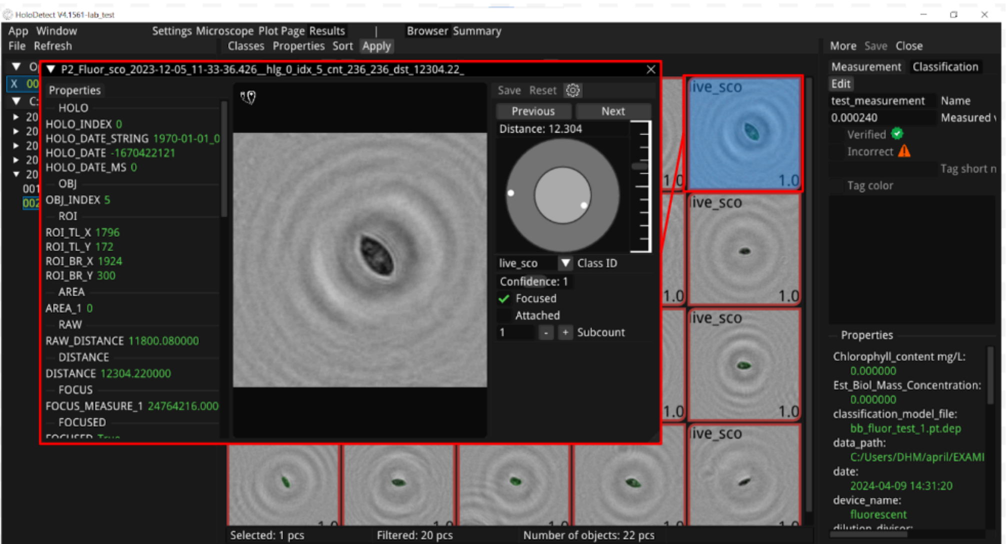
- Right-click on any object, and a list of possible activities will be displayed.
    - Click on the *Open in Editor* button to open the image.
    - You can reclassify it using the *Annotate selected* button.
    - Press the *Invert selection* button to invert the selection.
    - You can select all 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.
  - Any changes made in this small window must be saved using the *Save* button at the top of the window to take effect.

- 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.
  - Use phase: You can remove your object's background.
  - Show mask: marks everything that is in focus as an object in a given image.
  - Show fluor: Shows every fluor signal that is detected in that image.
  - Apply mask on fluor: If you tick this function and after use Show fluor too, you will see only the fluor signal of our object.
  - Apply a mask to the image: You can temporarily delete your object's background.

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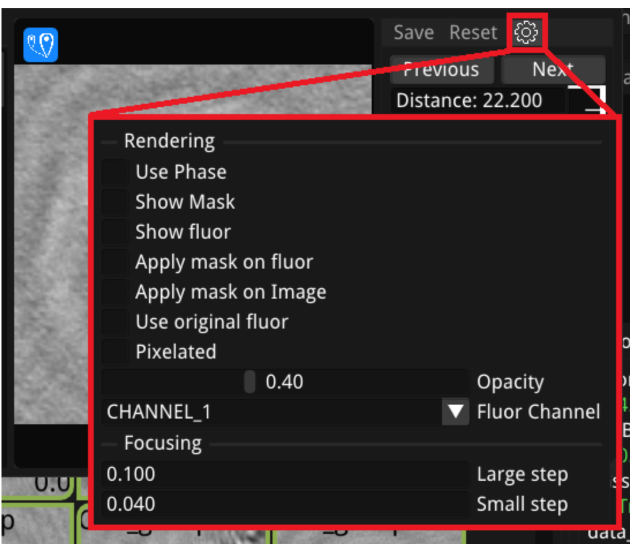
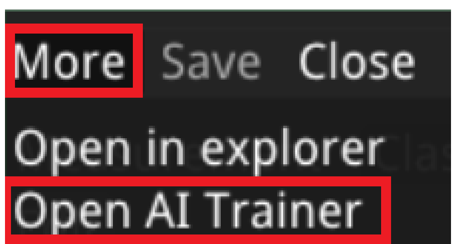
- Use original fluor: If you chose both this function and ‘Show fluor, you will see all three fluor signals at the same time.
- Pixelated: the image becomes pixelated.
- Opacity: You can set the intensity of the mask and the fluor mask.
- Fluor Chanel: Depends on what kind of laser is installed in the microscope. 3 channels are available , channel_1, channel_2, channel_3. 3 laser wavelengths: 405nm, 450nm, 532nm, and these channels are the absorptions given to the 3 lasers.

○ 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.



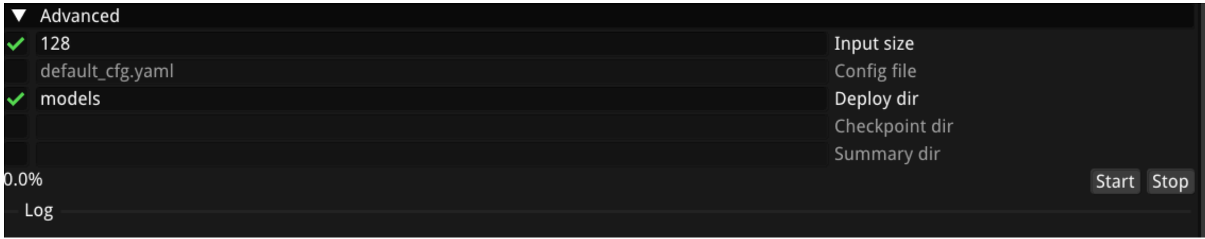
Using the AI-Trainer Learning Module

The AI trainer can be accessed by clicking the AI *Trainer* button under the *Window* tab, or by clicking the *More* button in the *Browser* window and selecting *Open AI Trainer*. 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 AI Trainer 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 AI Trainer Learning Module), and then name the model you are creating. The *System (algae/worm)*, *Device (CUDA/CPU),* and *Input size (128/256)* parameters do not need to be changed (default values: *System - algae*, *Device - cuda*, *Input size - 128*). System and input size depend on the type of microscope. They must match.

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.

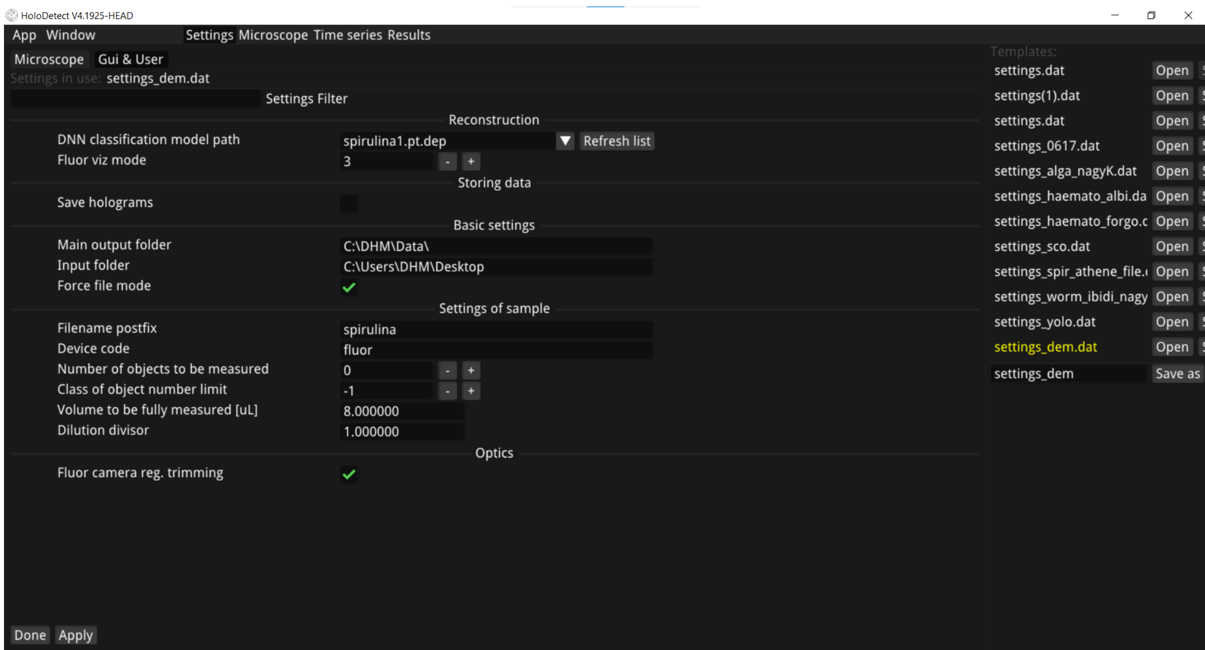
▶ Tuner ✕

Step-by-step measurement demonstration

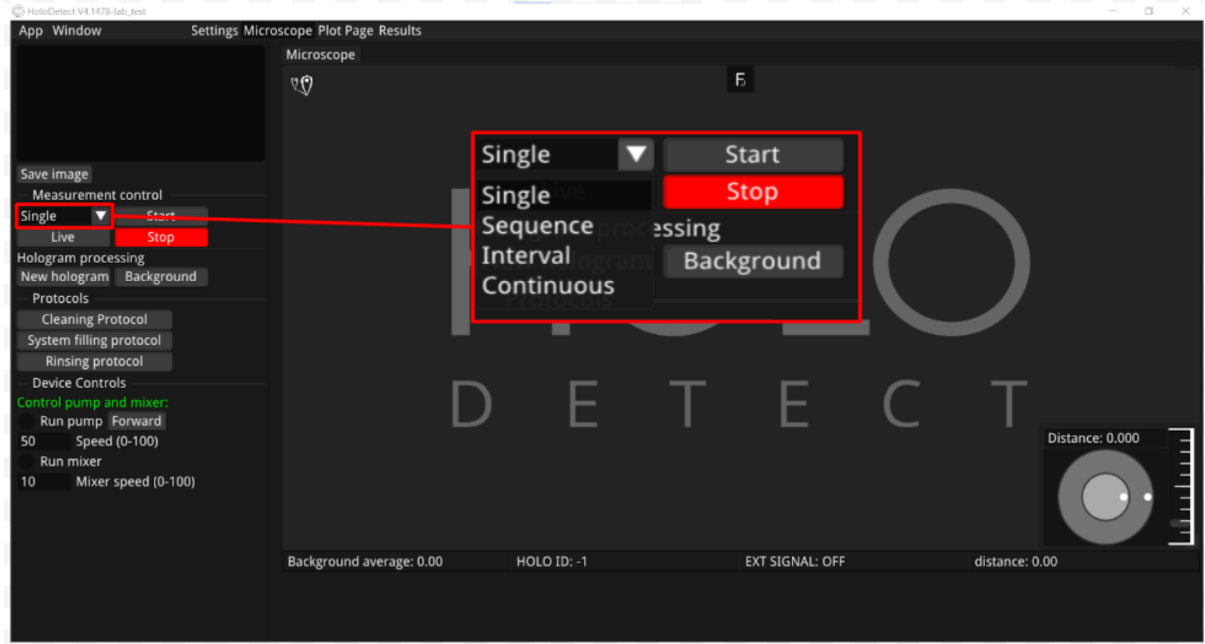
The following section describes the steps of a measurement and the evaluation of the results.

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. Next, specify the folder where you want to save the results and, if you are using File Mode, the folder from which you want to reprocess the holograms contained in it. In this case, you should also select Force file mode. Give a name to the measurement, name the device, and then set the volume to be measured. 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. After every setting, push the apply and done button.

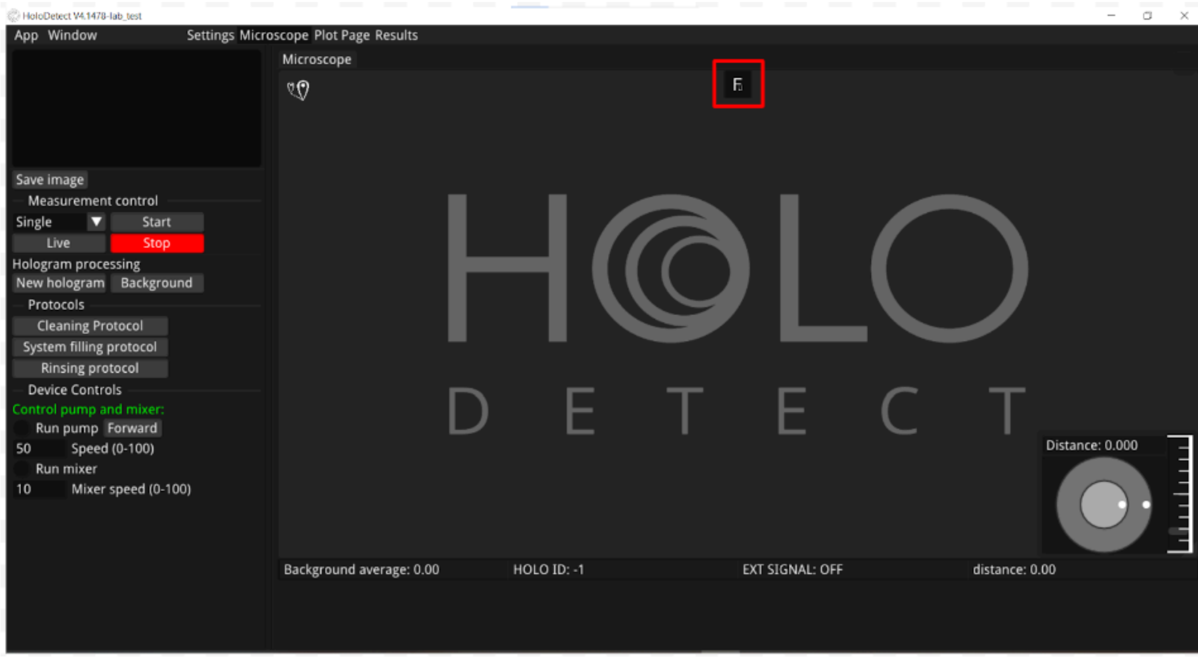
There are several buttons to the right of the Settings files: click on Use to open the file you have selected, click on S to save it, click on R to rename it, click on D to duplicate it, and click on X to delete it.

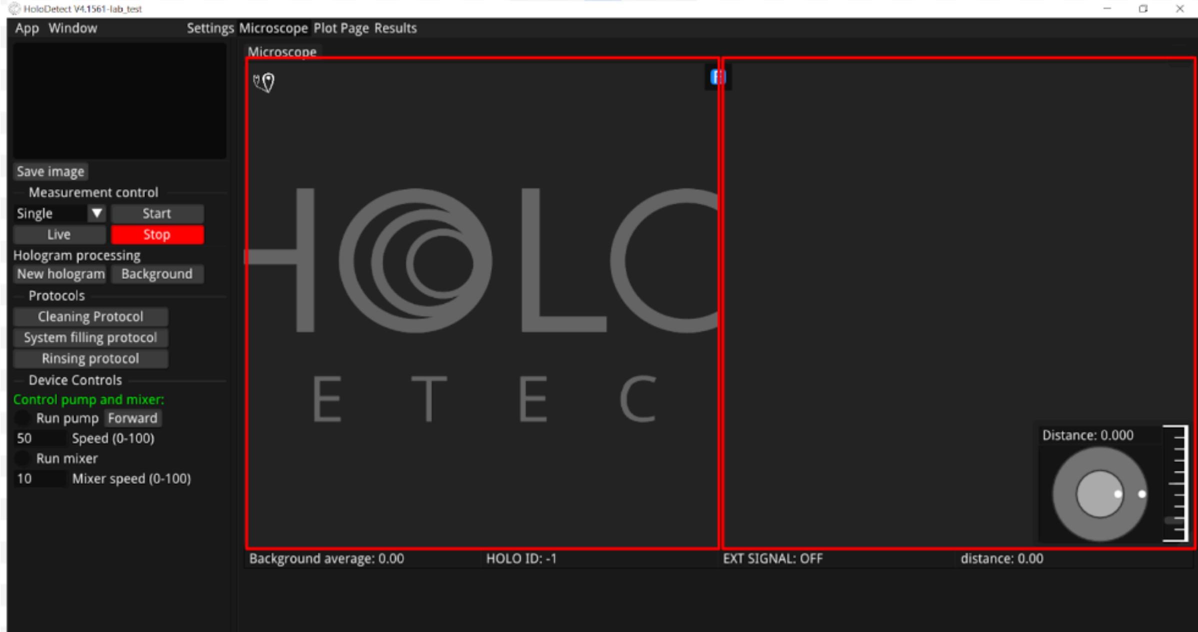


For measurements, you can choose from the four options already mentioned: Single, Sequence, Interval and Continuous.



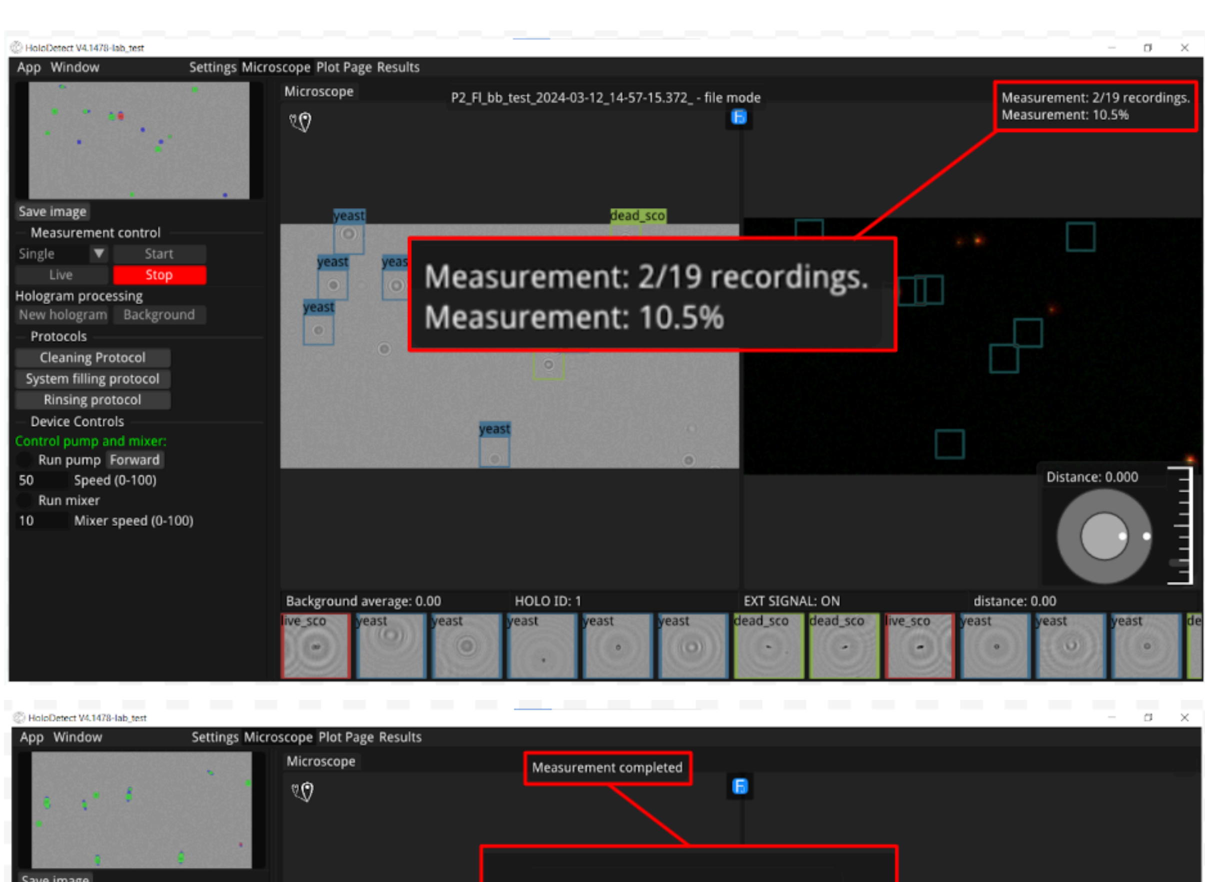
Press the Start button to start the measurement, then click the F button in the middle of the screen to display the fluorescence image and the hologram at the same time.

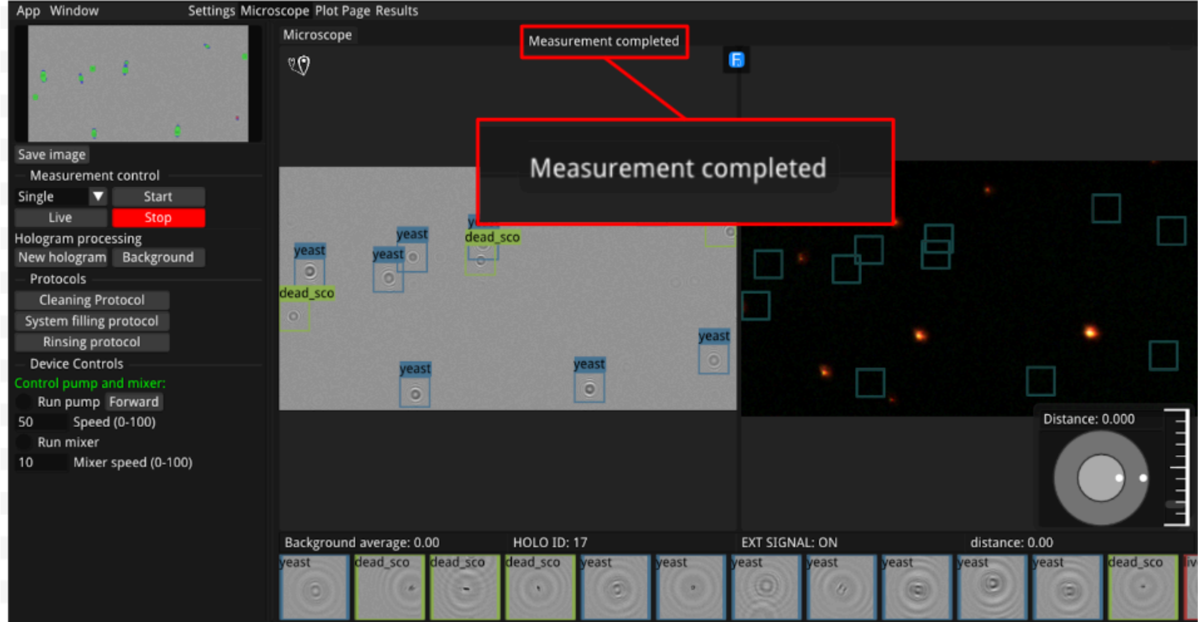




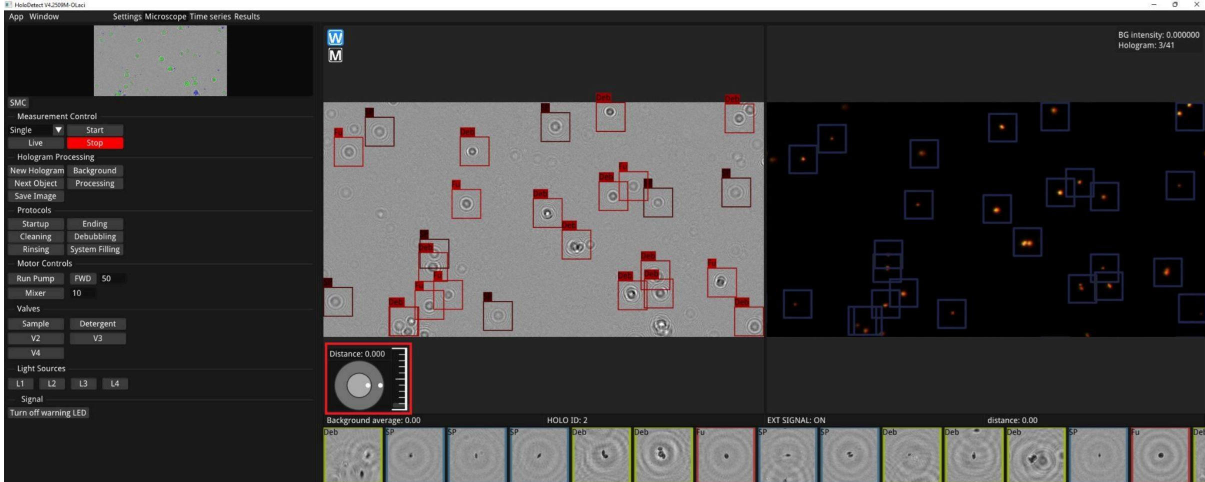
Double-click on the holographic image to display both the holographic and fluorescence images at full size.

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.

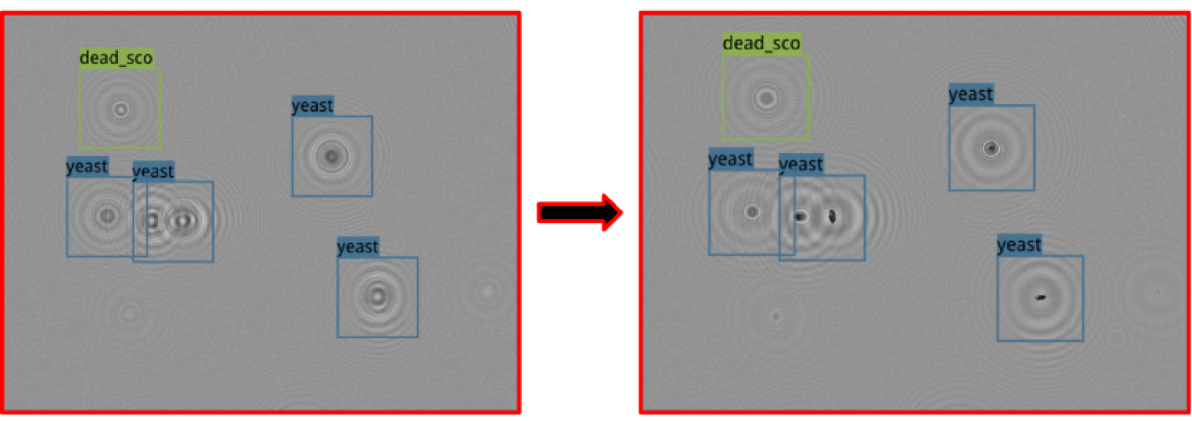




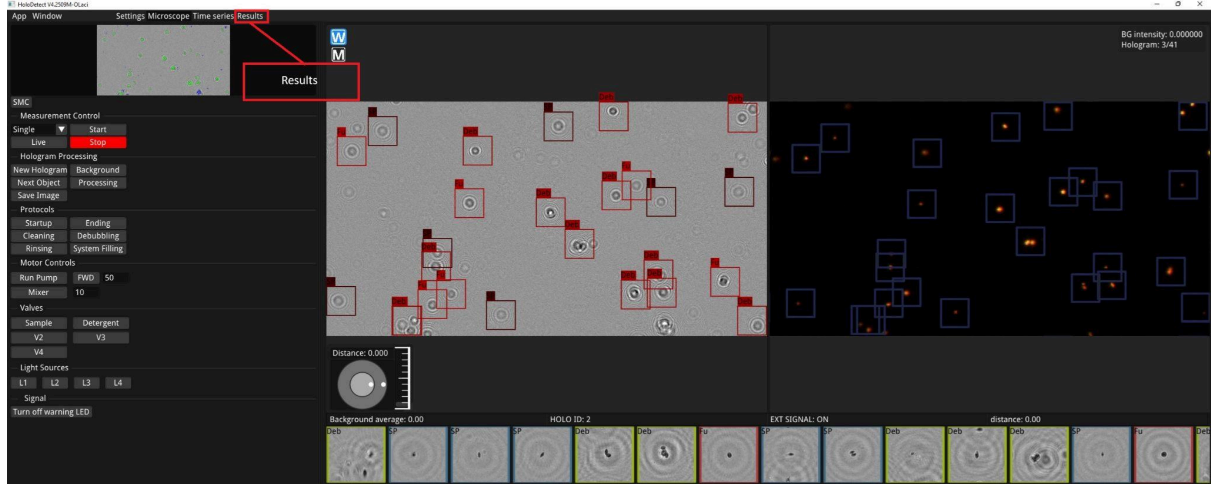
When you click on the New hologram button, stop a measurement in progress, or a measurement is finished and the last image is open, you have the option to focus the hologram or save the original or the refocused hologram by clicking on the Save image button.



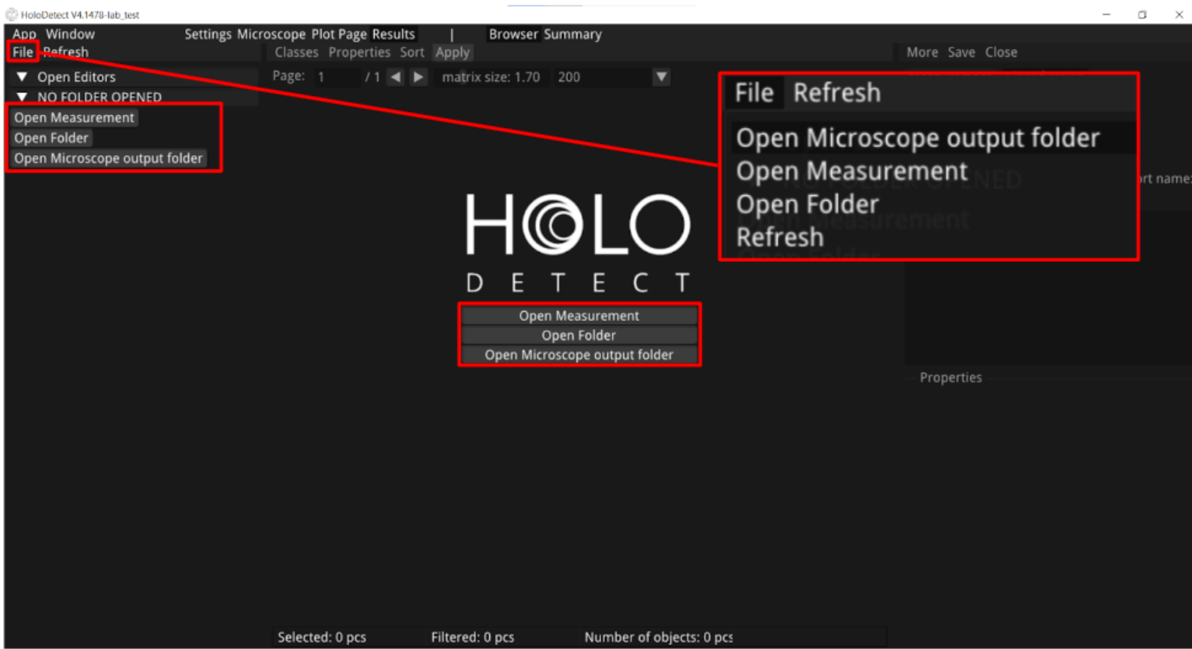
Focusing a hologram:



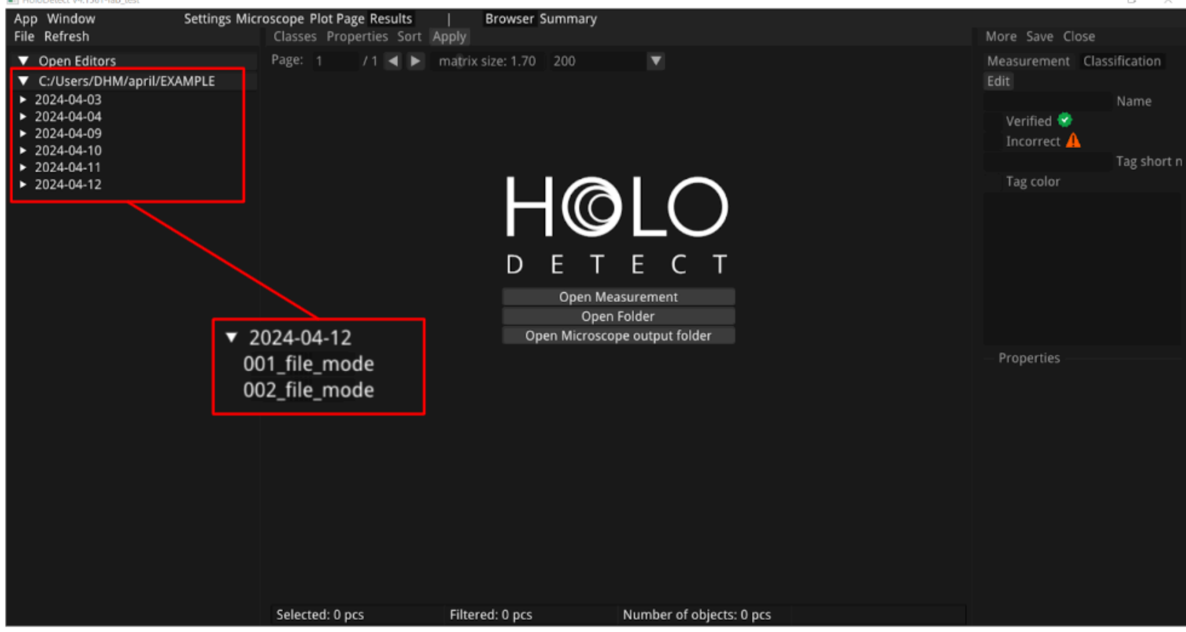
You can view the results for each measurement by pressing the Results button.

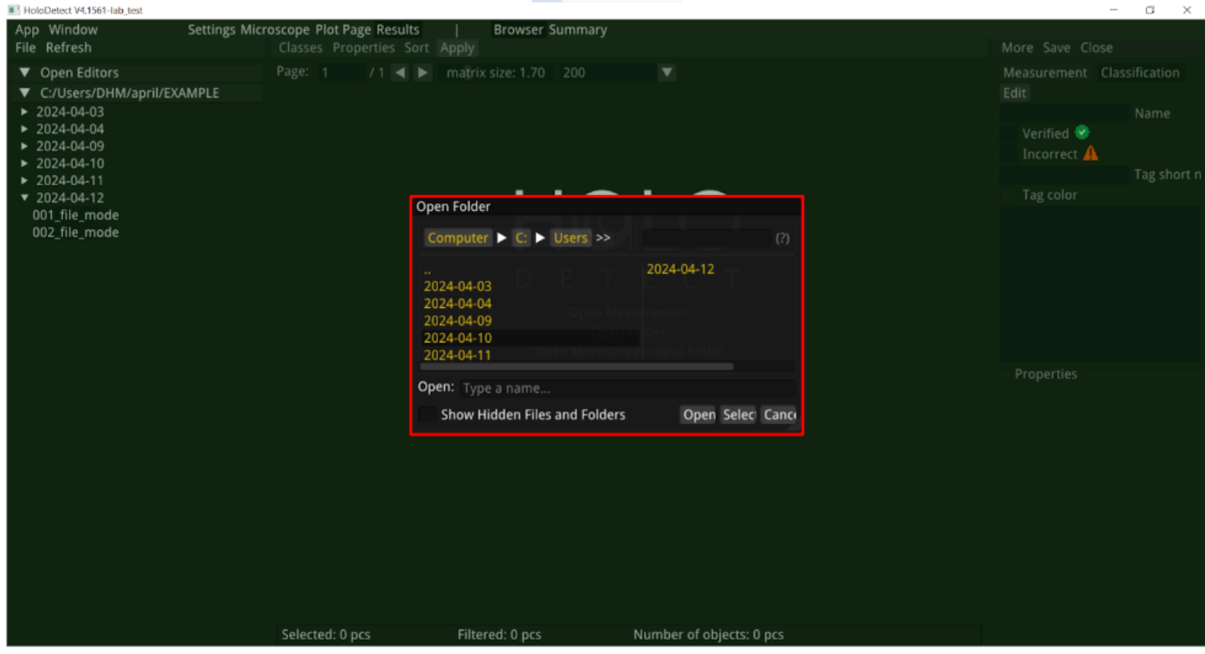


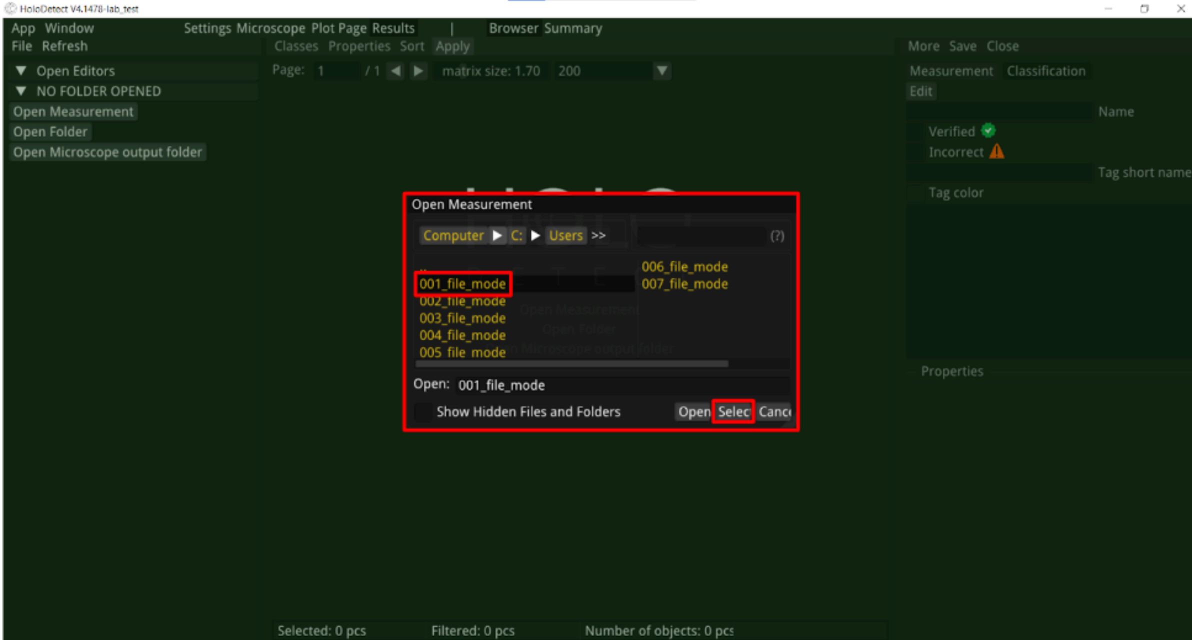
There are several ways to open the measurements: you can select the folder you specified in the Settings (Open Microscope output folder), or you can open a separate measurement (Open Measurement) or a folder of your choice (Open Folder).



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

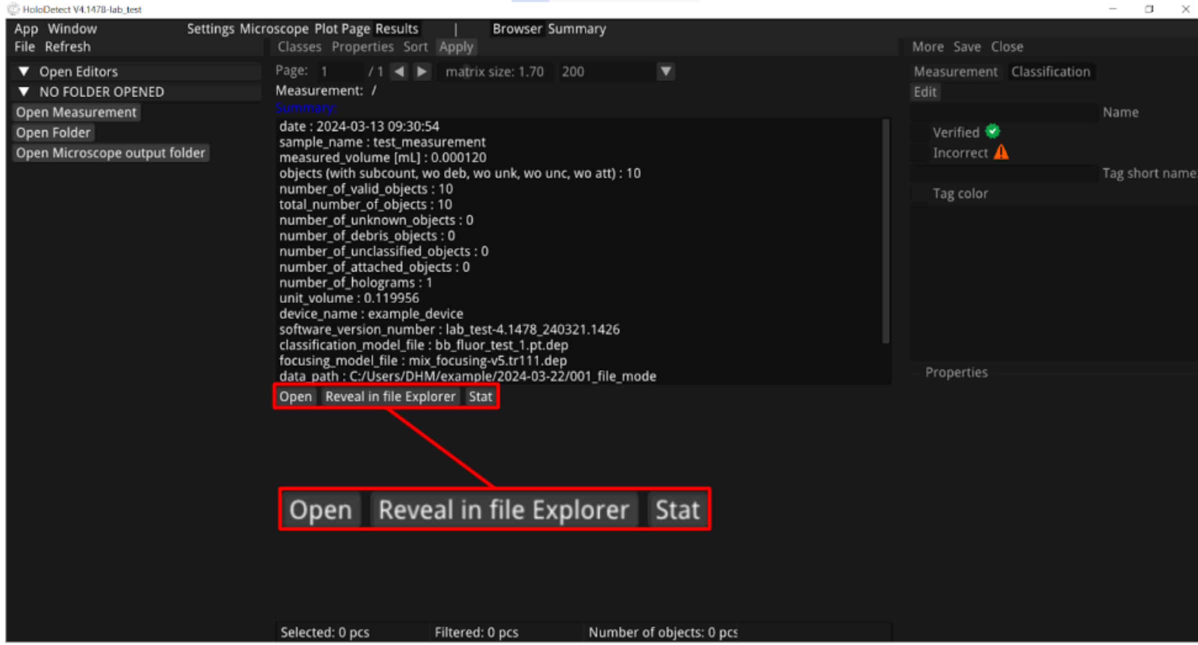




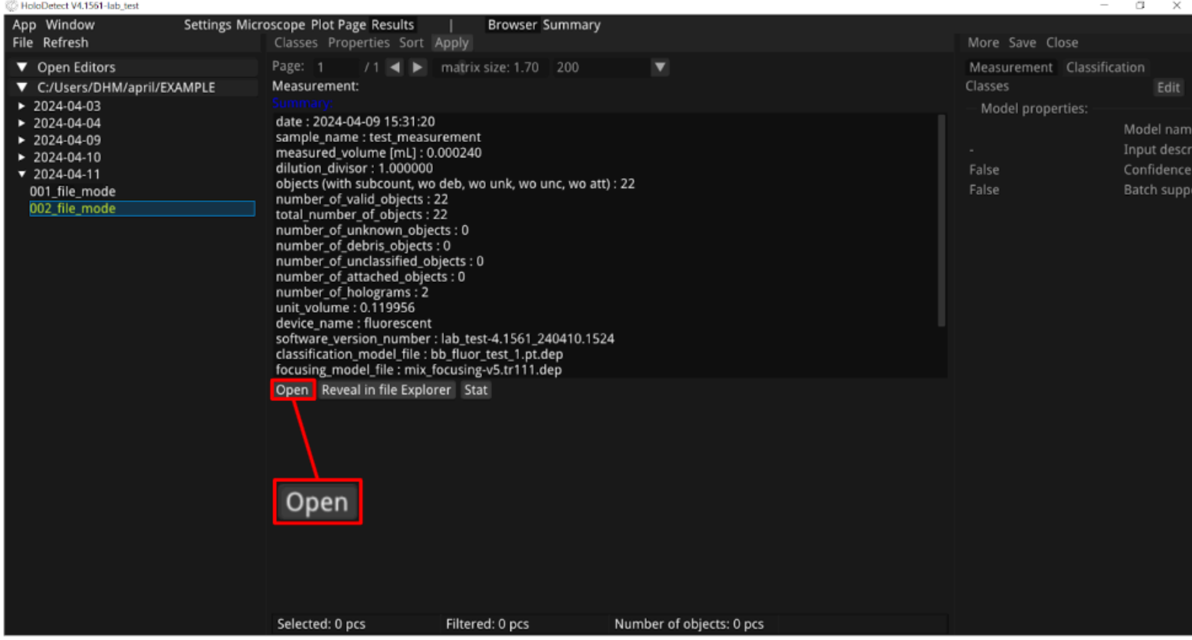


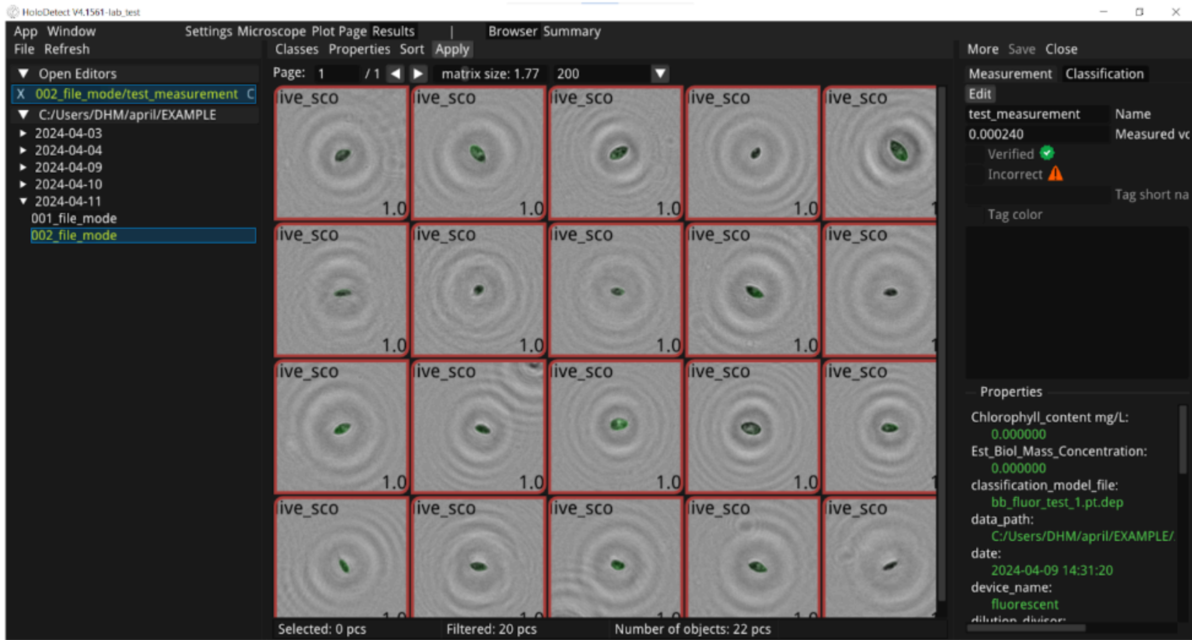
Double-clicking on the wo points exits this folder.
Once you click on it, you have three options to choose from:
1. Open the measurement (Open)
2. Open it in File Explorer (Reveal in File Explorer)
3. or open the statistics for the measurement (Stat).

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

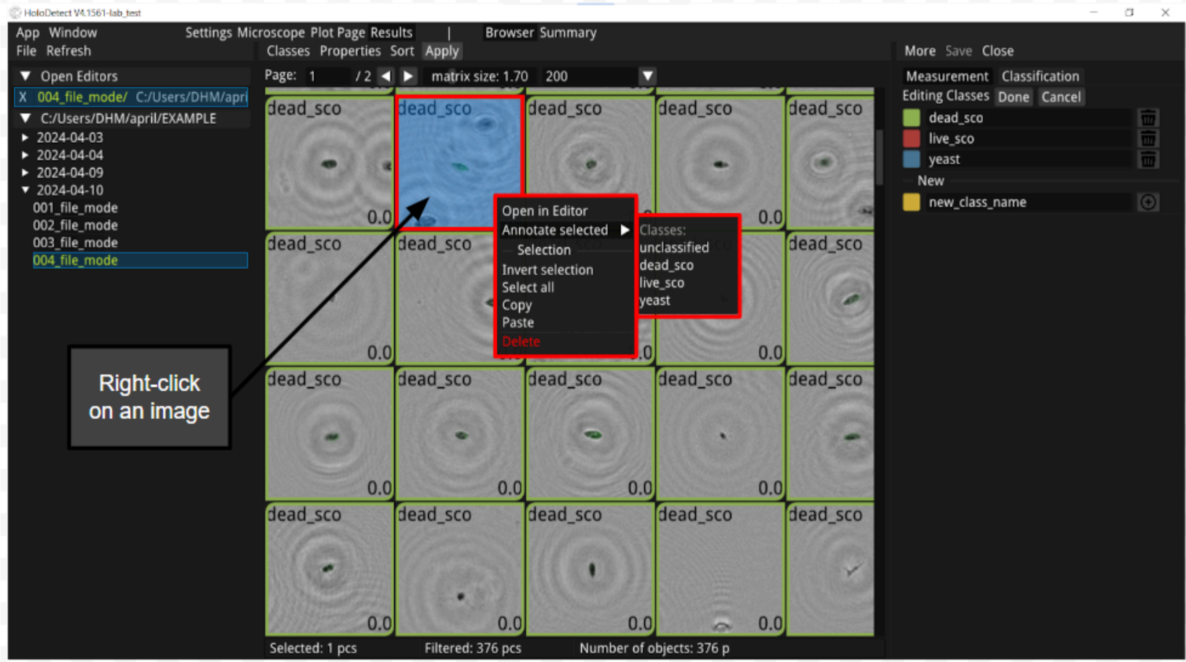


To view the measurement, select Open.

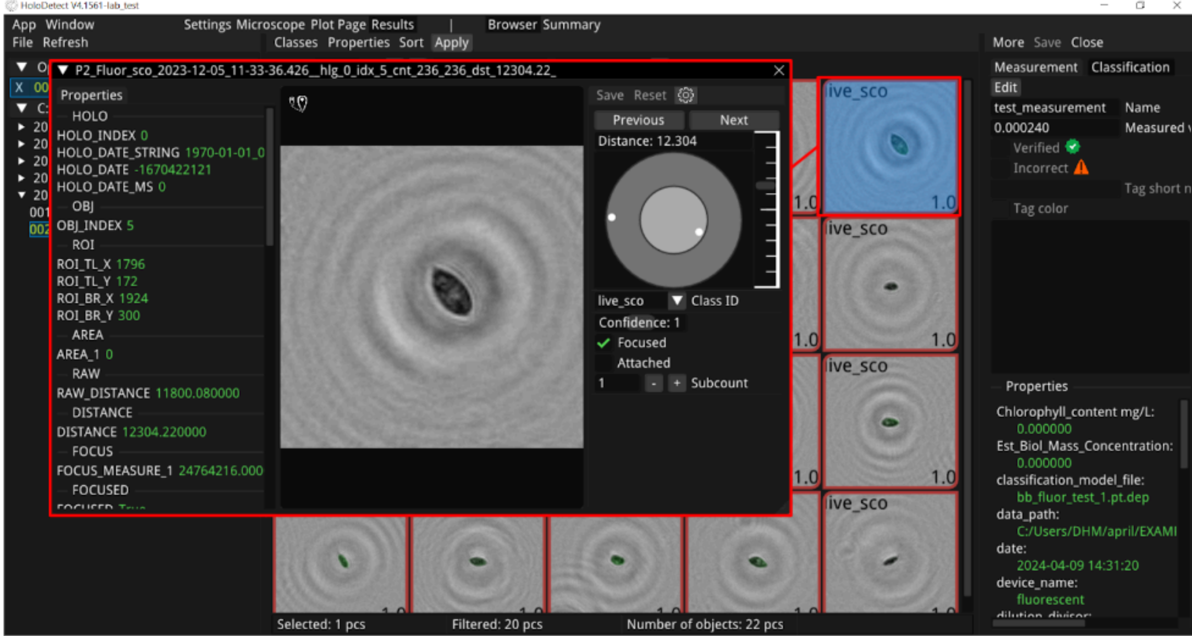




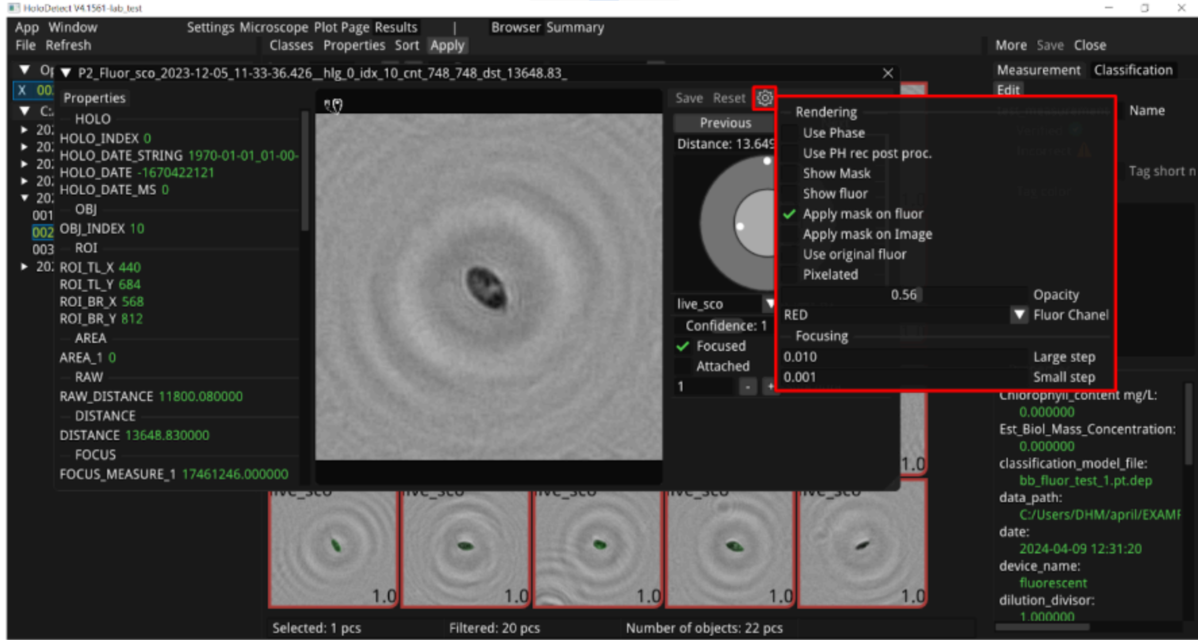
When you open a measurement, you will first see the objects found in the measured sample, sorted into classes. Select an object and right-click on it: a list will pop up offering several options. You can open the selected image (Open Editor), or you have the option to reclassify the selected object(s) 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.



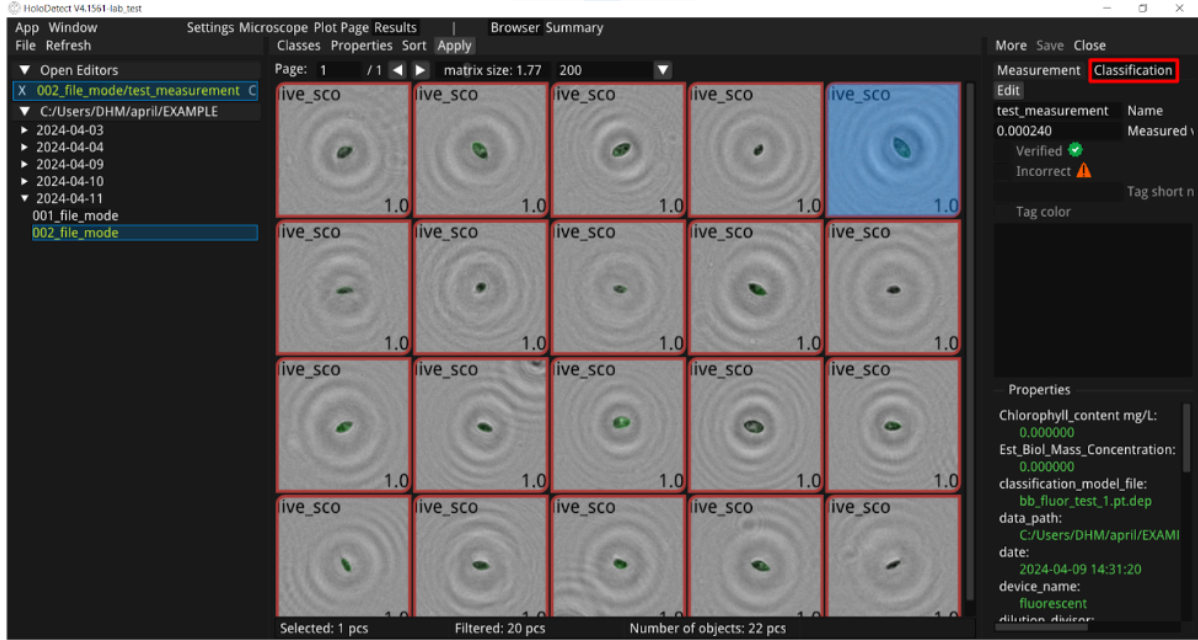
Select any image and press the Open in Editor option to open it. Here you can adjust the focus of the image using the sliders on the right, change the Class ID of the image, and adjust the number of cells in the image (Subcount) for a more accurate concentration count. You can select whether the opened 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.

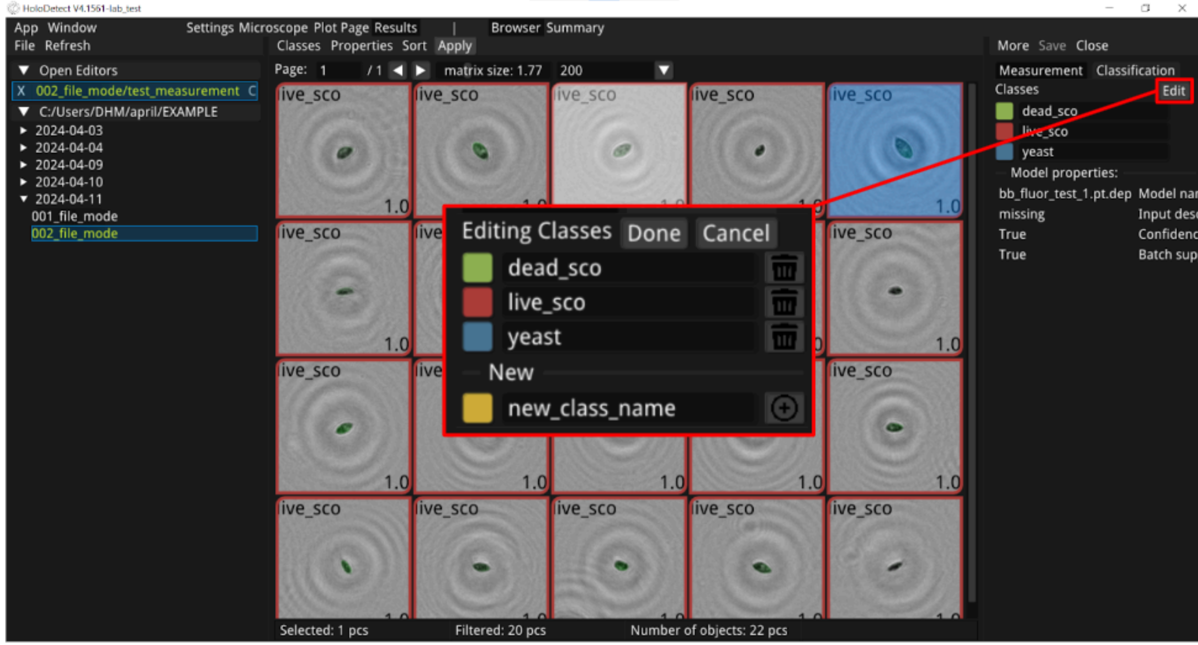


By clicking on the gear icon, you can choose which image to display:

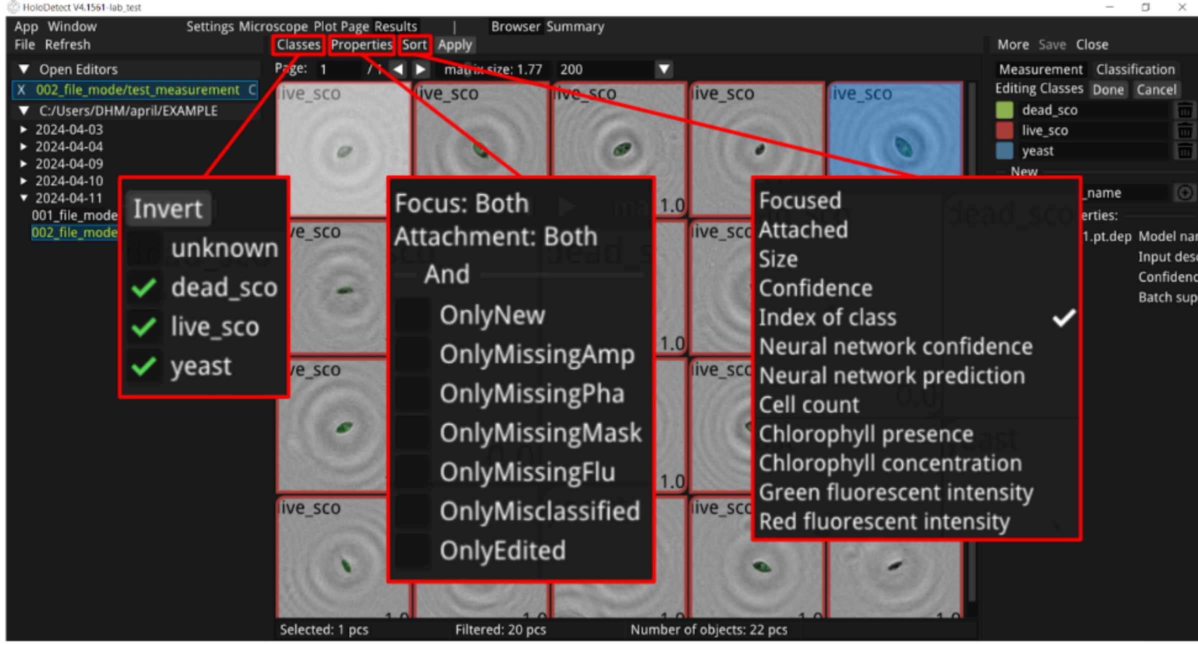


Clicking on the Classification tab on the right will display the currently existing classes and their corresponding colors. It is possible to create a new class and delete an existing class by first pressing the Edit button and then pressing the + or - icons. If you want to rename an existing class, 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

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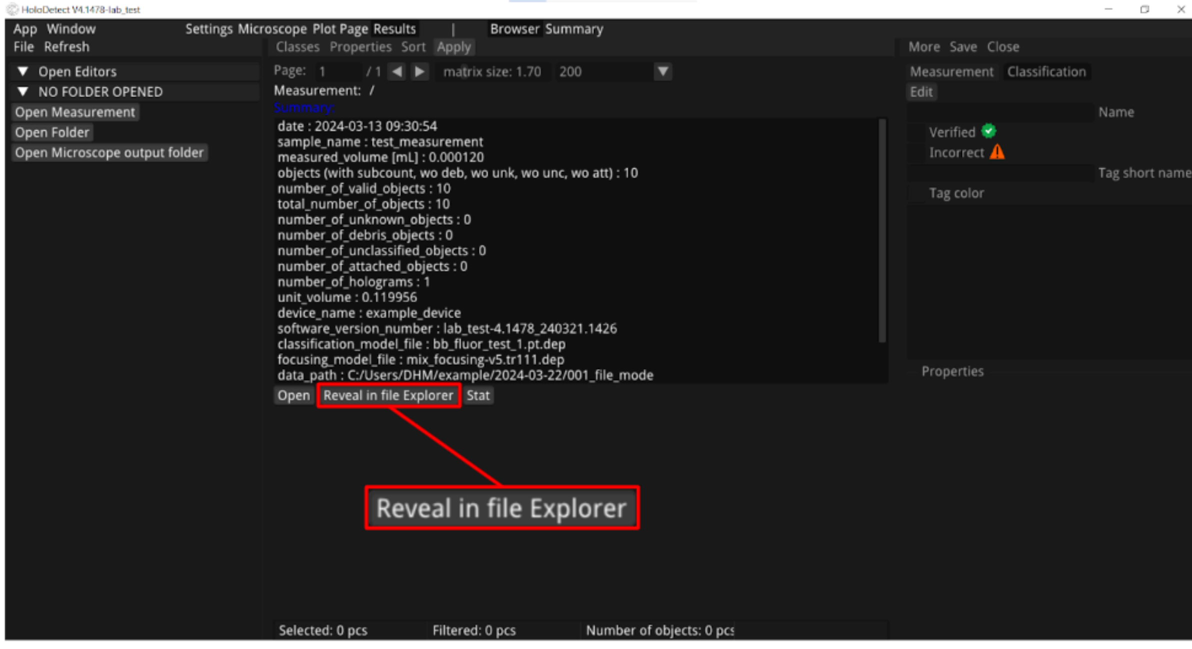
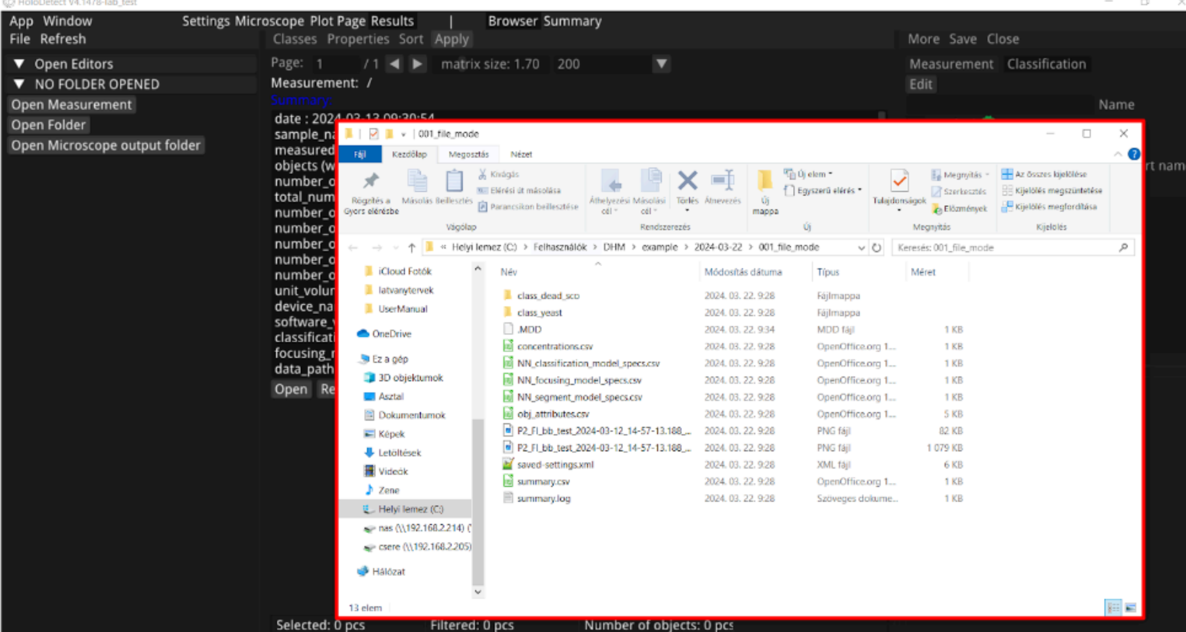
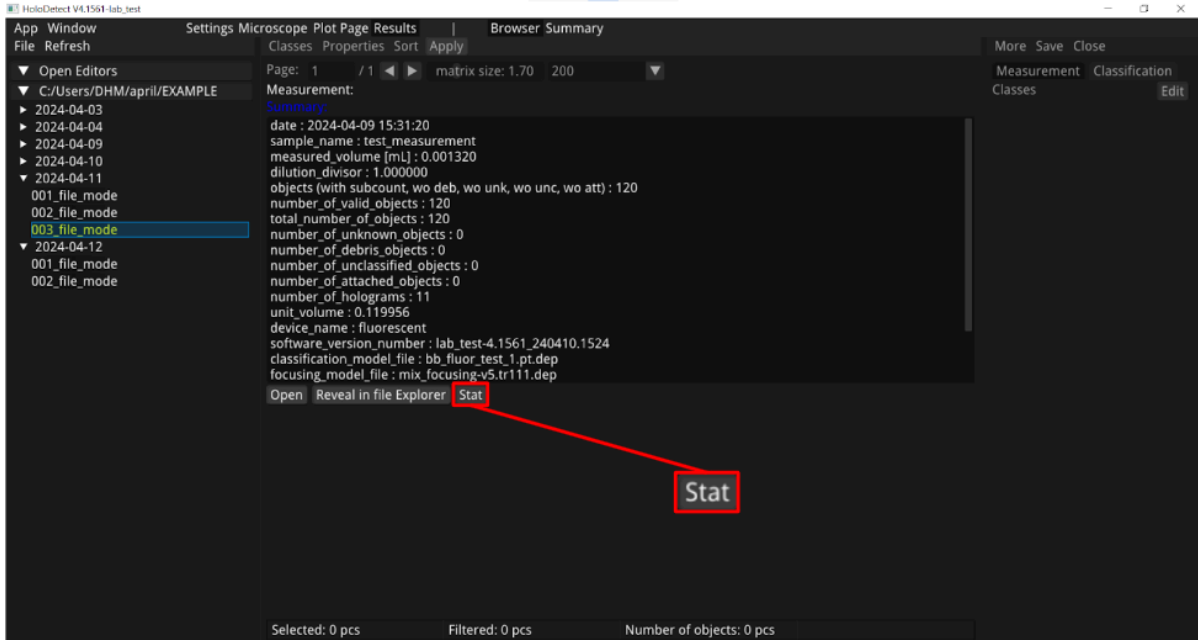
saving is enabled), the folders containing the objects, and the files describing the measurement results in the usual folder system.

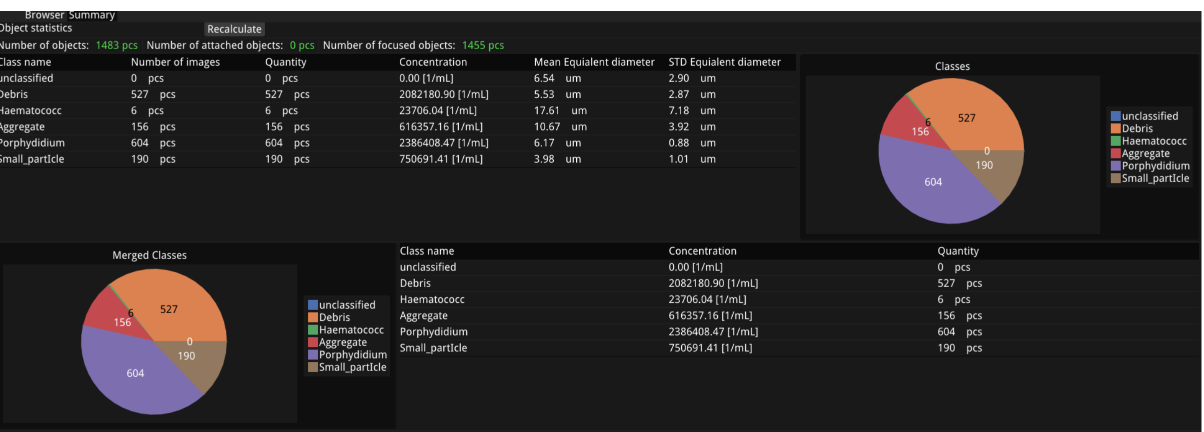




If you only want to open statistics for a specific measurement, you can do so by clicking on the Stat button when you select a measurement from the list on the left. The images are then not displayed, only the pie charts and tables containing the data.



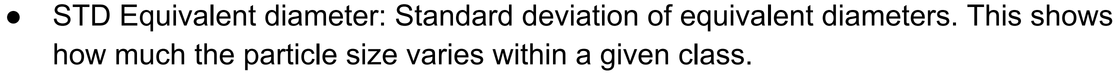
The first table will list each class separately, but if you have used the naming convention mentioned earlier (classname_p1, classname_p2, where the classname must be the same in all cases, followed by an underscore, then a letter p and a number), the second table will contain the combined values of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



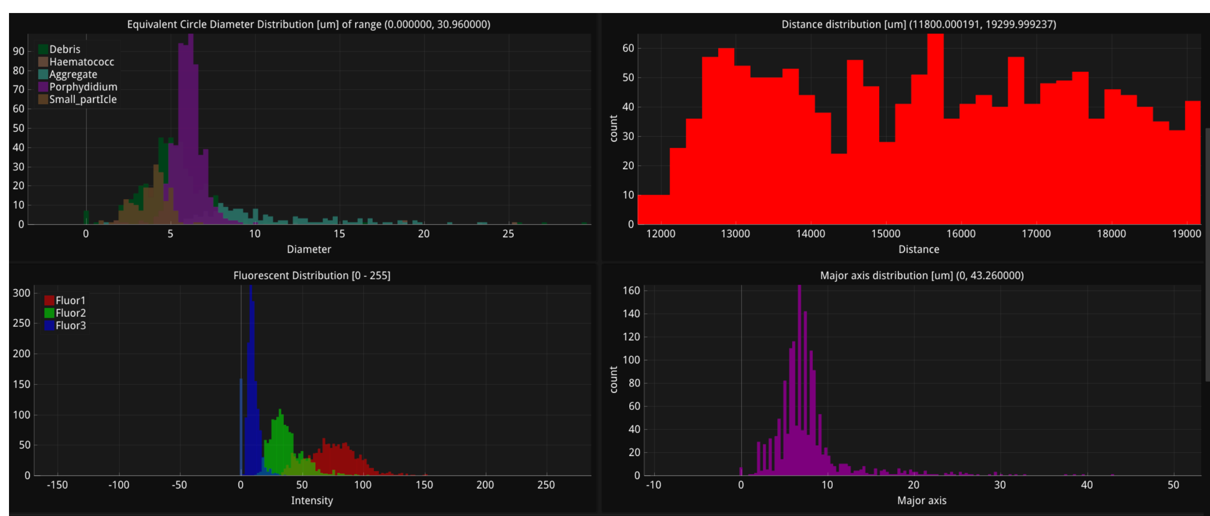
Class name	Number of images	Quantity	Concentration	Mean Equialent diameter	STD Equilent diameter
unclassified	0 pcs	0 pcs	0.00 [1/mL]	6.54 um	2.90 um
Debris	527 pcs	527 pcs	2082180.90 [1/mL]	5.53 um	2.87 um
Haematococc	6 pcs	6 pcs	23706.04 [1/mL]	17.61 um	7.18 um
Aggregate	156 pcs	156 pcs	616357.16 [1/mL]	10.67 um	3.92 um
Porphydidium	604 pcs	604 pcs	2386408.47 [1/mL]	6.17 um	0.88 um
Small_particle	190 pcs	190 pcs	750691.41 [1/mL]	3.98 um	1.01 um

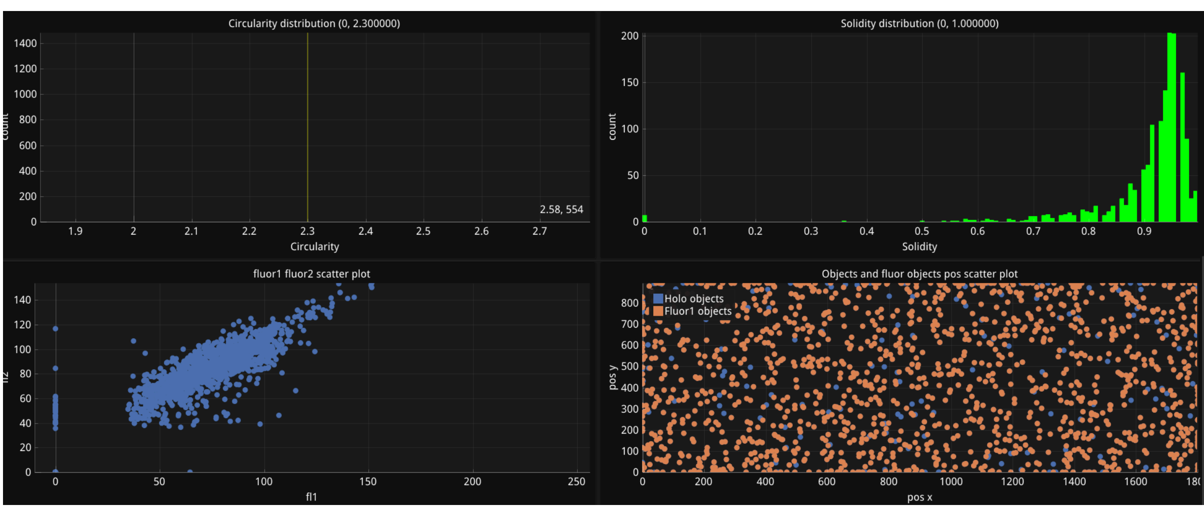
Class name	Concentration	Quantity
unclassified	0.00 [1/mL]	0 pcs
Debris	2082180.90 [1/mL]	527 pcs
Haematococc	23706.04 [1/mL]	6 pcs
Aggregate	616357.16 [1/mL]	156 pcs
Porphydidium	2386408.47 [1/mL]	604 pcs
Small_particle	750691.41 [1/mL]	190 pcs

- Class name: Every created class in your measurement, plus an unclassified one.
- Number of images: Number of images where that kind of object exists.
- Quantity: How many objects exist in those measurements.
- Concentration: The detected particle number is divided by the volume flowed through to obtain the concentration in units of 1/mL.
- Mean Equivalent Diameter: is the diameter of a circle that has the same area as the measured particle. Since particles are not perfect circles, the software calculates an "imaginary" diameter from the area and then averages it.



- STD Equivalent diameter: Standard deviation of equivalent diameters. This shows how much the particle size varies within a given class.





- There are eight bar charts at the bottom of the page:
  - equivalent circle diameter distribution (um) of range:

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This parameter represents the diameter of the circle that is part of the projected area of the particles – a circle that has the same area as the area shown in the image of the particle. It helps to analyze the polydispersity (multiple sizes) of the size distribution. If you click a class's name, you can display or make it disappear.

- Distance distribution (um)

  This shows the distribution of distances in μm (0–5000 μm), representing distances between particles, diffusion paths, or distances measured in aggregates as a histogram. Useful for studying aggregation or movement of objects.

- Fluorescent distribution (um)

  Fluorescence intensity distribution (in BI units, ~0–300), for three fluorophores (Fluor1 green, Fluor2 red, Fluor3 blue line).

- Major axis distribution (um)

  The major axis length in μm (0–5 μm) measures the length of elliptical or rod-shaped nanoparticles.

- Circularity distribution (um)

  It represents the size distribution (in pixels) of the objects found in the measurement.

- Solidity distribution (um)

  The second shows the distribution of the reconstruction depths (in micrometers) of the objects found in the measurement.

- Fluor1 fluor2 scatter plot

  The third shows the distribution of the measured fluorescence intensities of the objects of the measurement in the different color channels.

- The object and fluor object position scatter plot shows the distribution of fluorescent and non-fluorescent objects within the measured area. It can indicate the evenness of the illumination, and show if there are some masked areas within it.

Miscellaneous

For detailed *terms and conditions 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 a 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.

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- 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.

Appendix B

Description of the object attributes

The program saves the data of the measured objects in the object attributes.csv file.

HOLO_INDEX	HOLO_DATE_STRING	HOLO_DATE
HOLO_DATE_MS	OBJ_INDEX	ROI_TL_X
ROI_TL_Y	ROI_BR_X	ROI_BR_Y
BIG	CONC_FACT	AREA_1
RAW_DISTANCE	DISTANCE	DEPTH_3
FOCUS_MEASURE_1	FOCUSED	ATTACHED
CLASS_VAL	CLASS_CONF	FOCUS_X
FOCUS_Y	NN_FOCUS_VAL_1	NN_FOCUS_CONF_1
NN_CLASS_VAL_1	NN_CLASS_CONF_1	CUSTOM_CLASS_VAL_1
CUSTOM_CLASS_CONF_1	SUB_COUNT	MASK_AREA
MASK_NUMBER	SUBSEGMENT	MASK_SUMMEDAREA
MASK_MINENCCIRCDIAMETER	MASK_PERIMETER	MASK_ASPECTRATIO
MASK_MAJOR_AXIS	MASK_MINOR_AXIS	MASK_CIRCULARITY
MASK_COMPACTNESS	MASK_ELONGATION	MASK_RECT_AREA
MASK_EXTENT	MASK_EQUICIRCDIAMETER	MASK_LENGTH
MASK_THICKNESS	MASK_CENTER_POINT_X	MASK_CENTER_POINT_Y

MASK_IS_CONV	MASK_SOLIDITY	VOLUME_ESTIMATION
FLUOR_INTENSITY_RED	FLUOR_INTENSITY_GRE	FLUOR_INTENSITY_BLU
FLUOR1_AREAFLUOR1_SAT	CHLOROPHYLL_FLUOR	CHLOROPHYLL_CONT_FLUOR
FLUOR_2FLUOR2_INTENSITY_RED	FLUOR2_INTENSITY_GRE	FLUOR2_INTENSITY_BLU
FLUOR2_AREA	FLUOR2_SAT	FLUOR_3
FLUOR3_INTENSITY_RED	FLUOR3_INTENSITY_GRE	FLUOR3_INTENSITY_BLU
FLUOR3_AREA	FLUOR3_SAT	EXTERNAL_ENABLE_SIG
BUBBLE_SENSE	BG_CHANGE_SENSE	OBJ_FULL_NAME
MEASUREMENTS_YEAR_ID	FL1_POS_X	FL1_POS_Y
DIFF_HOL_FL1_POS_X	DIFF_HOL_FL1_POS_Y	DIFF_HOL_FL2_POS_X
DIFF_HOL_FL2_POS_Y	DIFF_HOL_FL3_POS_	XDIFF_HOL_FL3_POS_Y

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Appendix C

The files stored during a measurement

The program stores files after a measurement to ensure that all the information of the actual measurement is recorded. This can help to check, revise, or correct the measurement later using different settings parameters, like different calibration parameters, different NN classifier, and so on.

P2_alga_test_2026-02-18_18-33-57.749_hologram.png	The measured holograms. (Only if the user has approved in the settings, the hologram saving.)
P2_alga_test _2026-02-18_18-33-57.749_fluor.png	The measured 1st channel fluorescent images.
P2_alga_test _2026-02-18_18-33-57.749_flu2.png	The measured 2nd channel fluorescent images.
P2_alga_test _2026-02-18_18-33-57.749_flu3.png	The measured 3rd channel fluorescent images.
<DIR> class_SCEN_p1	The first classification directory
<DIR> class_sp	The 2nd classification directory
<DIR> class_debris	The 3rd classification directory
<DIR> …	Other classification directories
obj_attributes.csv	The attributes of the detected objects.
concentrations.csv	The measured concentrations.
Summary.csv	The short summary of the measurement.
NN_classification_model_specs.csv	Name of the classification model applied during the measurement.
NN_focusing_model_specs.csv	Name of the focusing model applied during the measurement.

NN_segment_model_specs.csv	Name of the segmentation model applied during the measurement.
summary.log	Short summary log.
saved-settings.xml	The settings file applied during the measurement.