

HOLOMONITOR® APP SUITE PROTOCOL

GENERAL CAPTURE ASSAY

This protocol helps to set up a General Capture Assay using HoloMonitor® M4 and the HoloMonitor® App Suite software. The HoloMonitor® Wound Healing Assay provides time-lapse images for further analysis by any other HoloMonitor® App Suite application.

REQUIREMENTS:

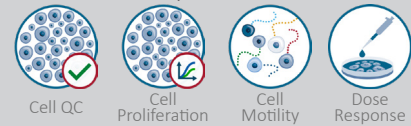
- HoloMonitor® M4, placed in incubator
- HoloMonitor® M4 App Suite
- Culture vessel of choice with cells
- HoloLid™ for selected vessel
- Vessel holder for selected vessel

OUTPUT:

- Selected data analysis depended

REANALYSIS:

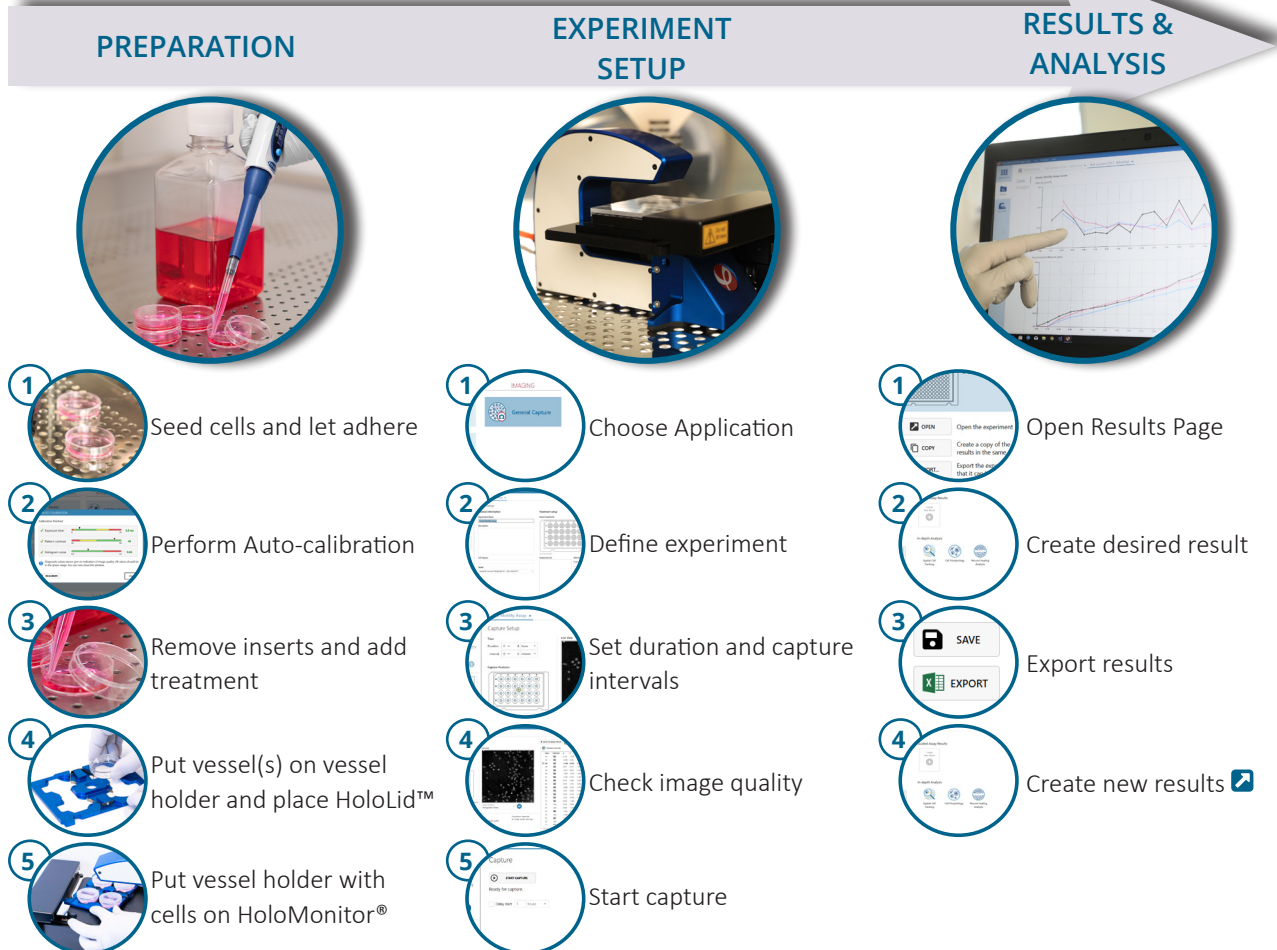
- Guided assays



- In-depth assays



WORK FLOW:



PREPARATIONS

Materials

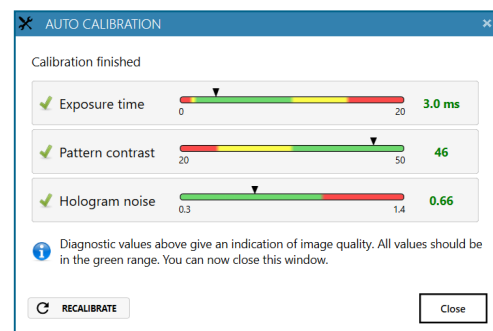
- ✓ HoloMonitor® M4, placed inside the incubator
- ✓ HoloMonitor® App Suite software
- ✓ Cell culture vessel. Please check our [list](#) with recommended vessels.
- ✓ HoloLid™ for the selected vessel
- ✓ Vessel holder for the selected vessel
- ✓ Cells
- ✓ [Setup and Operational Manual](#) for HoloMonitor®M4

Steps

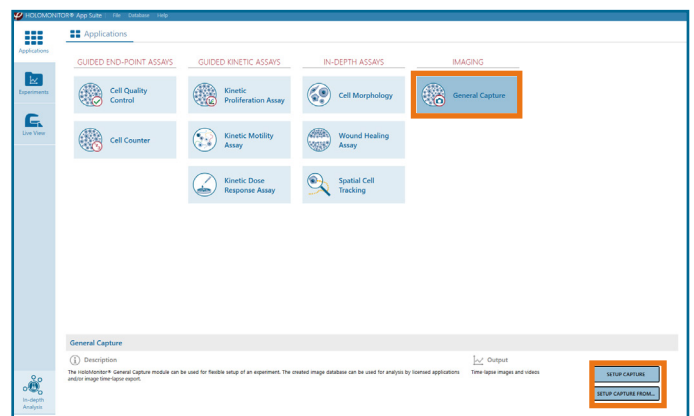
1. Seed the cells with about 5 % confluence (ca. 6000 – 11000 cells/cm²).
 - ▶ Please note that too few cells may lead to inadequate results due to auto-focus failure.
2. Place the vessel in the incubator and let cells attach for 2-24 hours.
3. Start the software and wait for complete instrument initialization.
4. Run an auto-calibration. With successful calibration, the instrument is ready to use.
5. Sterilize the HoloLids™ according to the specific [HoloLid™](#) protocol.
6. Add the treatment to your cells. The final working volumes per well, essential for using HoloLids™, are shown in the table:

Vessel	HoloLid™	Final volume	Growth area, cm ² /well
Sarstedt TC-dish 35 (cat. 83.3900)	71110	3.0 mL/well	8.0
Sarstedt TC 6-well plate (cat. 83.3920.005)	71120	3.0 mL/well	8.8
Sarstedt lumox® 24-multiwell plate (cat. 94.6000.014)	71130	1.8 mL/well	1.9
Sarstedt lumox® 96-multiwell plate (cat. 94.6000.024)	71140	170 µL/well	0.2
ibidi® µ-dish 35 mm, high (cat. 81156)	71111	2.5 mL/well	3.5
ibidi® µ-plate 24 Well Black (cat. 81156)	71131	2.5 mL/well	1.9

7. Slide the cell culture vessel onto the Vessel holder, its grips facing towards you. Ensure that the vessel is parallel to the holder. There is a spring that holds the vessel in place.
 - ▶ When using multi-well plates, place them with the cut-off corner to the left.
8. Replace the standard lids with the HoloLid™.
9. Put the vessel holder with the sample on the HoloMonitor® M4 stage and click it to secure.
10. Select the General Capture Application and proceed by clicking the Setup Assay button.



Successful auto-calibration window

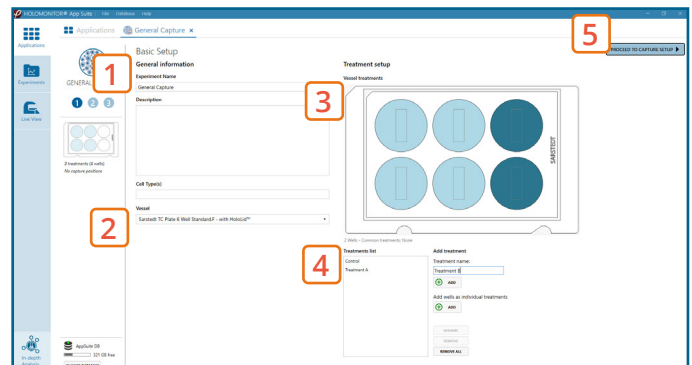


AppSuite main window with selected General Capture Application

EXPERIMENT SETUP

1 Basic setup: describe the experiment and assign treatments/conditions to the wells

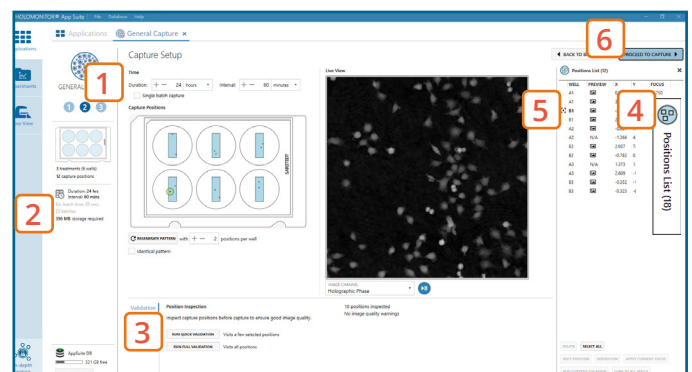
1. Enter the experiment name, optional experiment description and cell types.
2. Select the correct vessel map from the drop-down list.
3. Map treatments and conditions on the vessel map. Select wells by marking them with the left mouse button while moving the cursor over the relevant well/s.
4. Add the treatment name/s in the text box below the vessel map and click Add /press Enter. It is possible to add wells as individual treatments. Marked well/s are light blue, selected wells will appear dark blue.
5. Proceed to Capture setup.



Basic Setup window


2 Capture setup: Select the experiment time settings and choose capture positions

1. Adjust the default settings for duration, interval and number of positions.
2. Ensure that the storage requirement for the experiment does not exceed the computer capacity.
3. Run a full or quick validation of the selected positions to ensure good image quality.
4. Click the positions list button to view the position list.
5. Inspect the images by hovering over the image icon in the list. Move the stage to that position by double clicking the icon. If the image quality is poor, a warning sign ⚠ appears. Adjust position location or focus if necessary.
6. When satisfied with the experiment setup, click Proceed to Capture.



Capture Setup window

XY-position



Focus

Step Length: 10 µm

Target

X: 94.263 mm
Y: 11.616 mm
Z: 1.595 mm

Actual

X: 94.263 mm
Y: 11.616 mm
Z: 1.595 mm

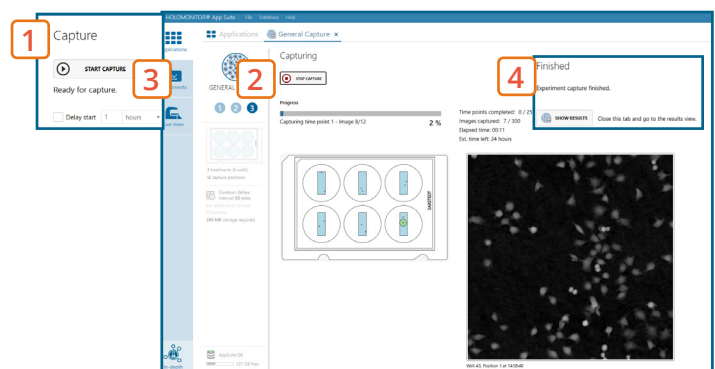
Software Focus

Focal distance (µm): 1351

MANUAL FOCUS ADJUSTMENT: The focusing tool is located in the Controls tab. Move the black square or click on the Arrow buttons to move the stage up and down. Save an adjusted focus setting for the selected position by using the Apply Current Focus button. For details, consult the Setup and Operational Manual.

3 Capture: Review the experiment in real-time during the time-lapse

1. Click Start Capture.
2. To stop the experiment ahead of time, click the stop button. Note that it is NOT possible to restart the experiment once it has been stopped.
3. Go to the Experiments tab and open your ongoing experiment to preview the captured images during the run.
 - Wait for the experiment to finish before starting data analysis.
4. When the Experiment capture finishes, click the Show Result button to get directly to the Results page.

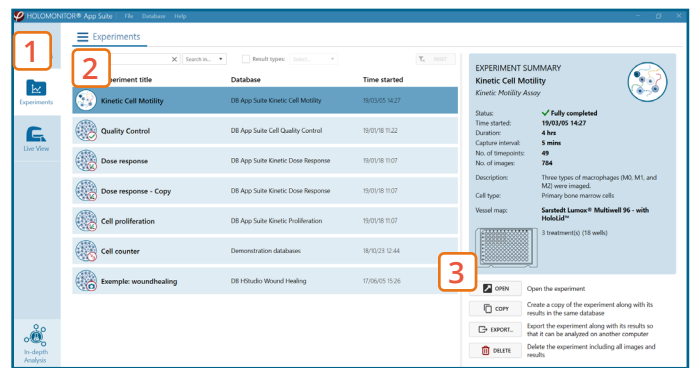


Capture window

RESULTS & ANALYSIS

Experiments tab

1. Click Experiments to see a list of the experiments.
2. Click on the experiment title to open an experiment summary.
3. Click **Open** to open the results page.



Experiments tab

Experiment overview tab

1. See the experiment summary, view all images and go to the experiment setup by choosing the respective tab.
2. Create **Guided Assay Results** from this experiment by clicking the button.
3. Generate **in-depth** analysis data from the captured images by clicking on the respective icon.
 - For further data analysis steps, please see the respective assay protocol.



Experiment overview tab

The HoloMonitor® App Suite experiment data can be re-analyzed to generate results for other assay types. Find more information in the [overview](#) and the [Setup and Operational Manual](#).