HOLOMONITOR® APP SUITE PROTOCOL

GENERAL CAPTURE

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:

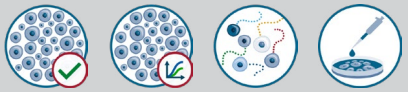
- HoloMonitor® M4, placed in incubator
- HoloMonitor® M4 App Suite
- Culture vessel of choice with cells
- HoloLid™ for selected vessel
- Vessel holder for selected vessel

OUTPUT:

- Selected data analysis depended

REANALYSIS:

- Guided assays



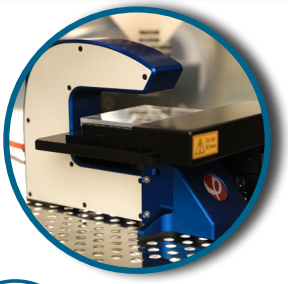
- In-depth assays

WORK FLOW:

PREPARATION

1. Seed cells and let adhere
2. Perform Auto-calibration
3. Remove inserts and add treatment
4. Put vessel(s) on vessel holder and place HoloLid™
5. Put vessel holder with cells on HoloMonitor®

EXPERIMENT SETUP



1. Choose Application
2. Define experiment
3. Set duration and capture intervals
4. Check image quality
5. Start capture

RESULTS & ANALYSIS

1. Open Results Page
2. Create desired result
3. Export results
4. Create new results

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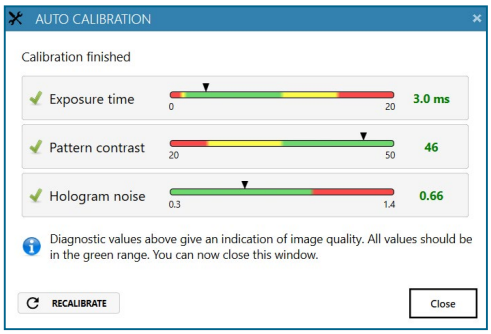
PREPARATIONS

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 Operation Manual** for HoloMonitor ®M4

Steps

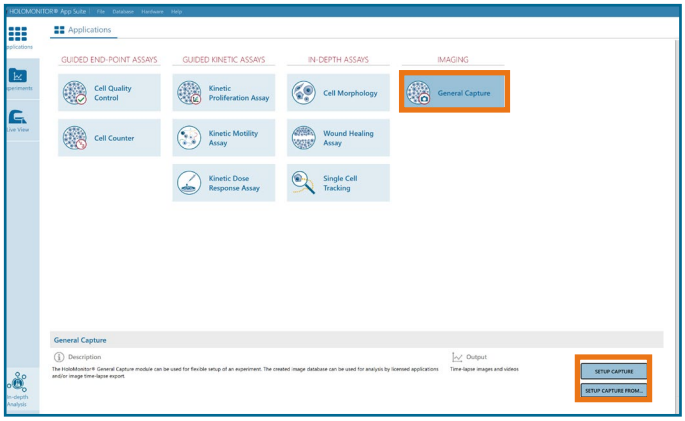
1. Seed the cells with about **5 % confluence** (ca. 6000 – 11000 cells/cm²).
   ►Please note that too few cells may lead to inadequate results due to auto-focus failure.
2. Place the vessel in the incubator and let **cells attach** for 2-24 hours.
3. **Start** the **software** and wait for complete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



Successful auto-calibration window

Vessel	Vendor cat. number	HoloLid™	Final working volume	Growth area, cm²/well	Vessel cut in a holder
Sarstedt TC-dish 35	83.3900	71110	3.0 mL/well	8.00	NA
Sarstedt TC 6-well plate	83.3920.005	71120	3.0 mL/well	8.80	top left
Sarstedt lumox® 24-multiwell plate	94.6000.014	71130	1.9 mL/well	1.90	top left
Sarstedt lumox® 96-multiwell plate	94.6000.024	71140	170 µL/well	0.34	top left
ibidi® µ-dish 35 mm, high	81156	71111	2.5 mL/well	3.50	NA
ibidi® µ-plate 24 Well Black	80241	71131	2.5 mL/well	1.90	NA
Eppendorf CCCadvanced® FN1- 6 well	0038110010	71150	3.0 mL/well	9.40	bottom right

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.**
10. Select the **General Capture Application** and proceed by clicking the **Setup Capture** button.

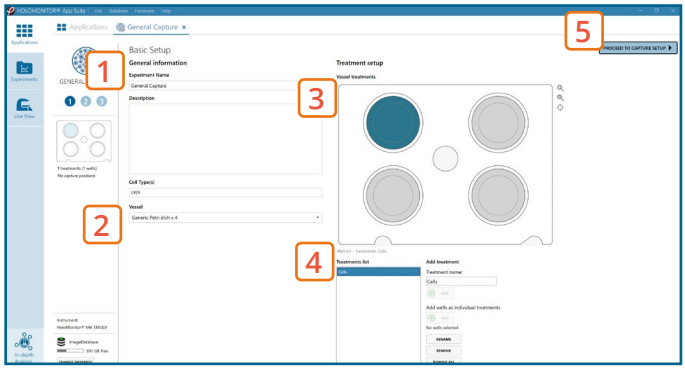


AppSuite main window with selected **General Capture** application

EXPERIMENT SETUP

1 Basic setup: describe the experiment and assign treatments 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 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**.

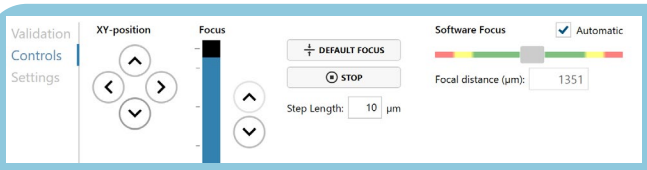


Basic Setup window

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.
   ► When running an experiment, **data needs to be stored on the computer** connected to the instrument. Storing data on an external drive (e.g. connected via USB or internet server) may cause data loss due to erratic USB connections or poor internet connection.
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**.

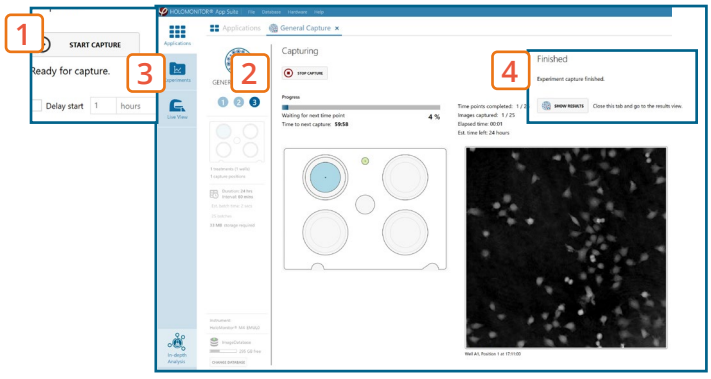
Capture Setup window



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 Operation 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.
   ► 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**.

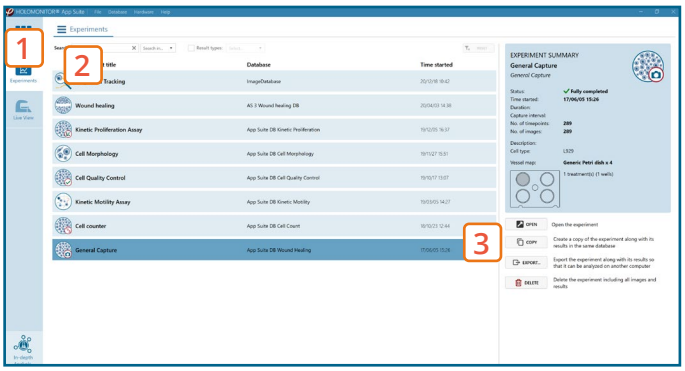


Capture window

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 to view all **images**.



Experiments tab

One experiment — multiple results

✓ This section helps to reanalyse data between different applications using HoloMonitor® App Suite software.

Generating In-depth Assay results

1. In the **Experiment overview page** select the In-depth application icon for wanted result.
2. **Follow** the respective assay protocol .

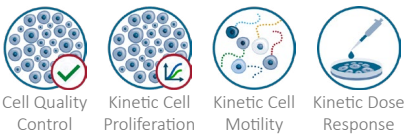
Generating Guided Assay results

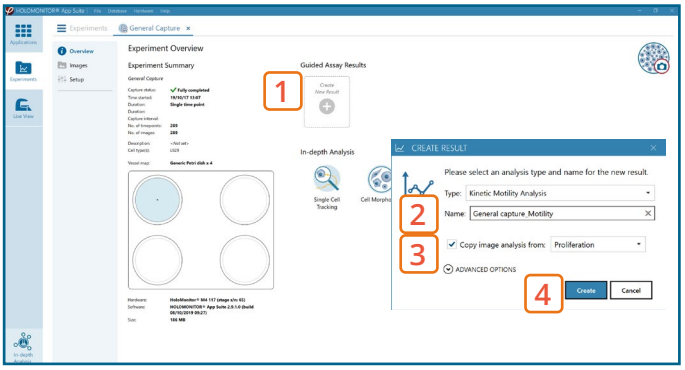
1. In the **Experiment overview page** under Guided Assay Results select **Create New Result**.
2. **Choose type** of analysis in the pop up window and **name** the new result.
3. Tick **copy image analysis from** and **select** the experiment to copy from. This will copy the image analysis settings from the selected result including all changes.
   ► For further data analysis steps, please see the respective assay protocol .
4. **Press create**.

Obtain these results from the General Capture data:

In-depth assays

Guided assays





Experiment overview tab

When **creating a New Guided Assay** result from **General Capture** experiment - the **first** analysis will take some time, as the software needs to evaluate image quality and perform image analysis.