

HOLOMONITOR® APP SUITE PROTOCOL

CELL MORPHOLOGY ASSAY

This protocol helps to set up a Cell Morphology Assay using HoloMonitor® M4 and the HoloMonitor® App Suite software. The HoloMonitor® Cell Morphology Assay enables non-invasive live-cell quantification of a wide range of morphological properties, including individual cell volume, area and thickness.

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

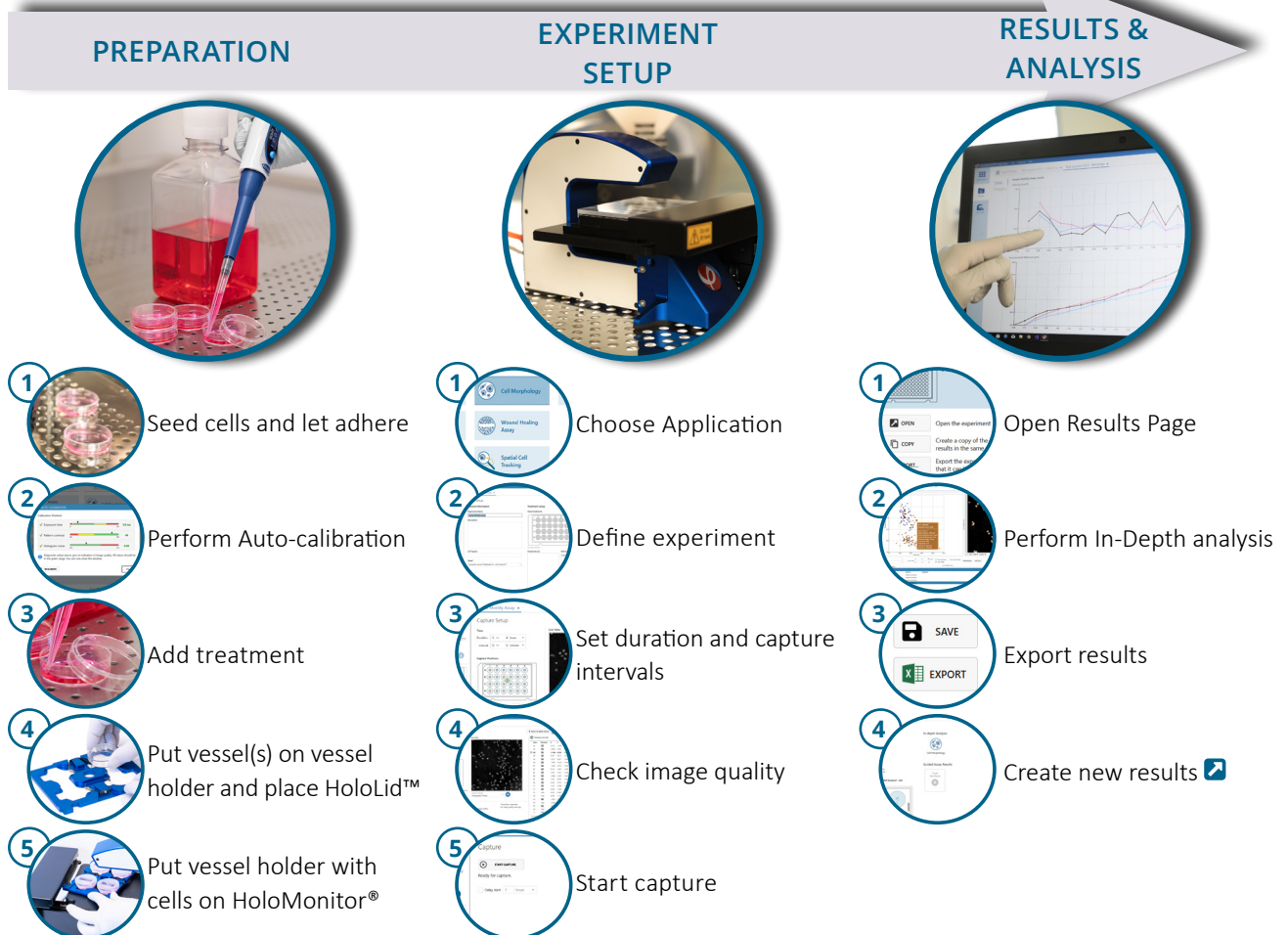
OUTPUT:

- 30+ parameters
- Calculation spread sheets of all data, including population averages

REANALYSIS:

- Guided assays
 - Cell QC
 - Cell Proliferation
 - Cell Motility
 - Dose Response
- In-depth assays
 - Spatial tracking
 - Cell Morphology

WORK FLOW:



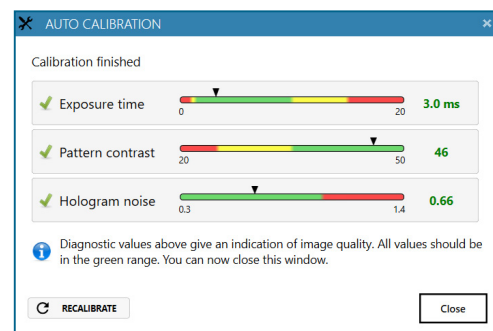
PREPARATIONS

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

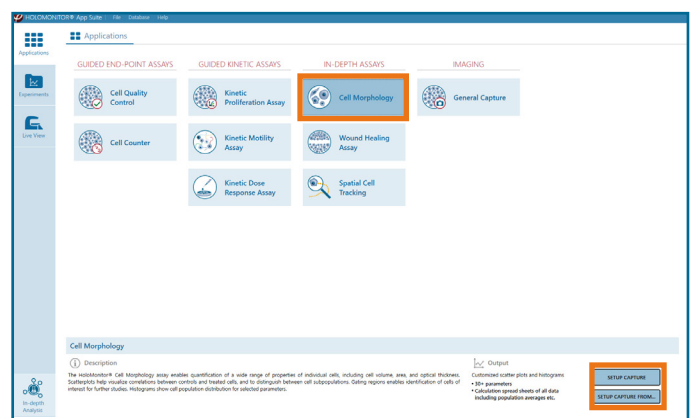
1. Seed the cells with about **5 % confluence** (ca. 6000 – 11000 cells/cm²).
 - ▶ Please note that too few cells may lead to inadequate results due to auto-focus failure.
2. Place the vessel in the incubator and let **cells attach** for 2-24 hours.
3. **Start the software** and wait for complete instrument **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:



Successful auto-calibration window

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 **Cell Morphology Assay** and proceed by clicking the **Setup Assay** button.

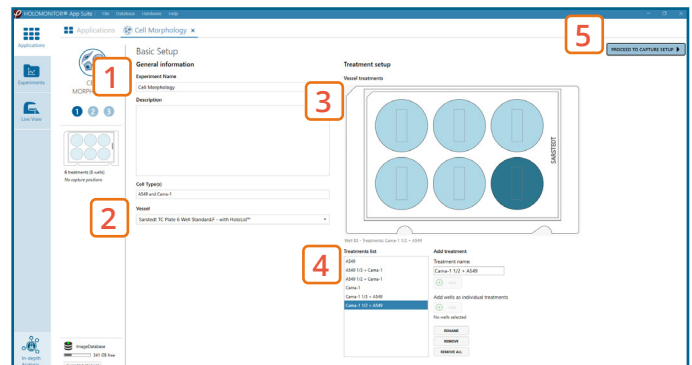


AppSuite main window with selected Cell Morphology Assay

EXPERIMENT SETUP

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

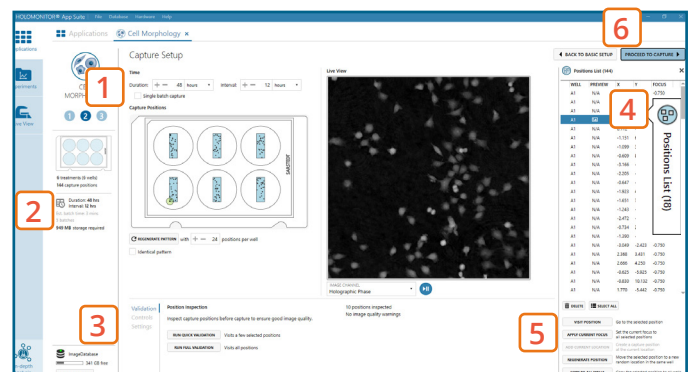
1. Enter the experiment **name**, optional experiment **description** and cell types.
2. Select the correct **vessel map** from the drop-down list.
3. Map **treatments and conditions** on the vessel map. **Select wells** by marking them with the left mouse button while moving the cursor over the relevant 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**.

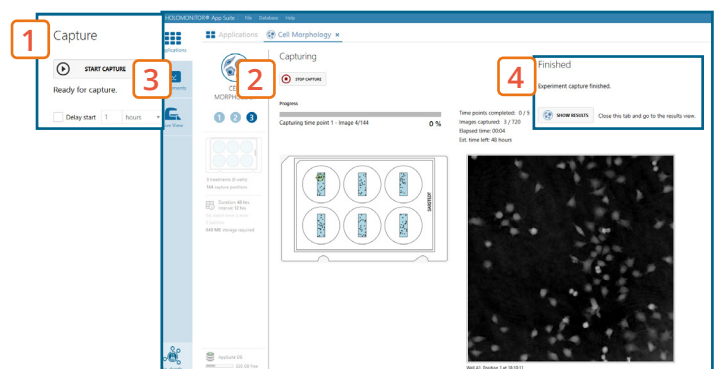


Capture Setup window

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 In-depth Analysis.
4. When the Experiment capture finishes, click the **Show Result** button to get directly to the **Results** page.

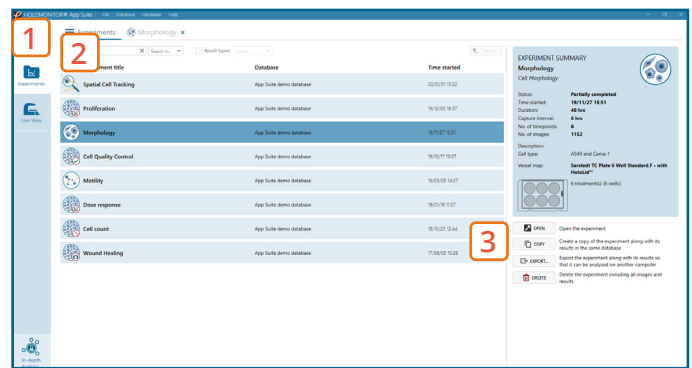


Capture window

RESULTS & ANALYSIS

Experiments tab

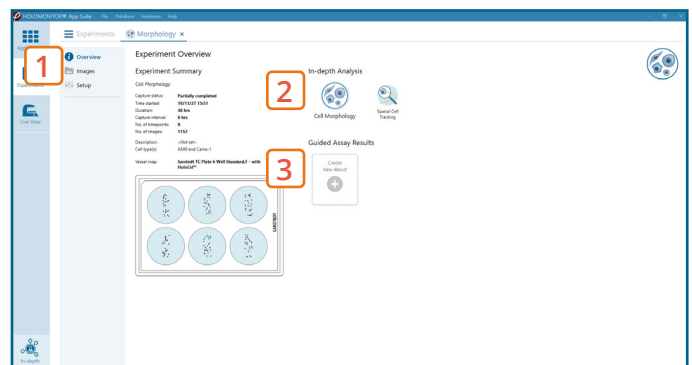
1. Click Experiments to see a **list of the experiments**.
2. Click on the experiment title to open an **experiment summary**.
3. Click **Open** to open the results page.



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. Generate **in-depth analysis** data from the captured images by clicking on the **Cell Morphology** icon. A **new window** for the in-depth analysis will open.
3. **Create New Guided Assay Results** from this experiment by clicking the respective button.

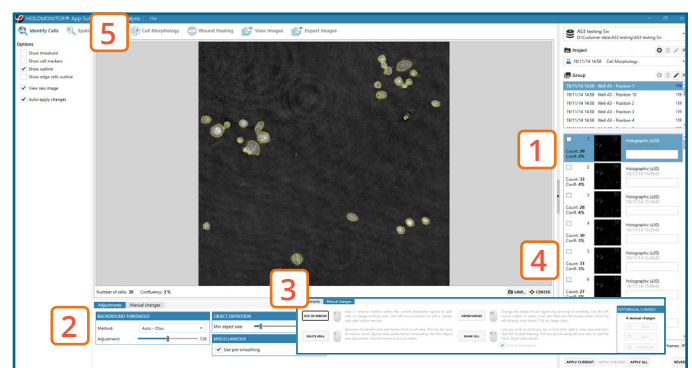
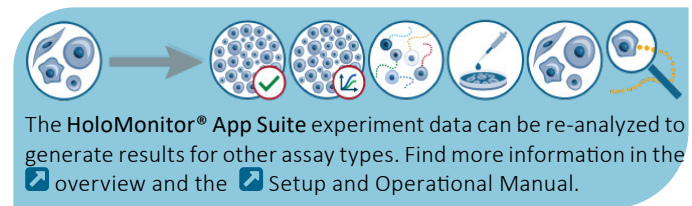


Experiment overview tab

In-depth analysis — identify cells tab

- ✓ First step for morphology analysis is to identify the cells.
- ✓ The software automatically calculates cell count and confluence of each image.
- ✓ Review the image quality and include/ exclude images from results analysis. See the [Image guide](#) for more information.

1. Start by selecting a **frame of interest**, so the image is displayed. You can alter the viewing options.
2. **Adjust** the background **threshold** and **object size** so the mask best fits the cell segmentation and click **Apply** (Current/ Checked/ All) to fix the settings to the frames and to display the **automatic calculation** of cell count (**#/frame**) and **confluence (%)**.
3. **Manual changes** are possible. Add/remove or grow/shrink a cell, delete a cell area or draw a cell area yourself. These changes will **only** be saved to the **currently selected image**.
4. **Check** cell segmentation for some images in the beginning, middle and end of the experiment to make sure the applied threshold fits. Scrolling through the image list and checking for abnormal jumps in cell count or confluence values may help as well to see if adjustments are necessary.
5. Once satisfied with the cell identification, **click** on the **Cell Morphology** tab to start morphology analysis.

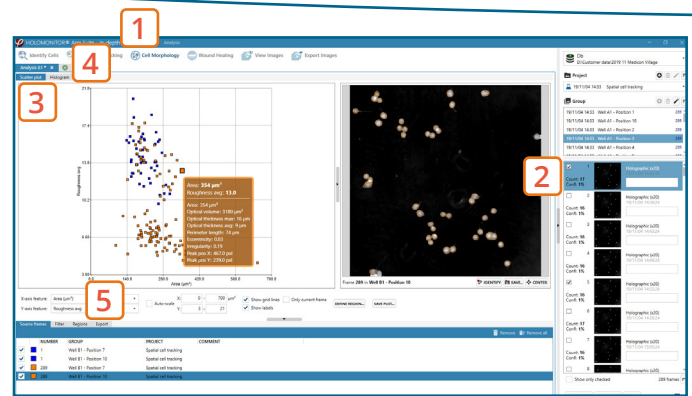


Identify cells tab

In-depth analysis — cell morphology tab

- ✓ Review the image quality and include/ exclude images from results analysis. See the [image guide](#) for more information.
- ✓ For further analysis all results can be exported.
- ✓ For more details, see the [Setup and Operational manual](#).

1. Click **New Analysis** to start a new data analysis or load a previous one by clicking the Open button.
2. **Add one or several frames** from the list to the analysis either by drag-and-drop or using the Add Selected/ Checked/ All button. You can sort the frames in the list by time or position.
3. **Morphology data** from all added frames is presented in a **scatter plot** where you can change the parameters of the x- and y-axis via the drop-down list. You can save the plot and alter scale, grid lines and label viewing options, too.
 - ▶ The selected source frame is shown in the **image**. Hovering over a marked cell or a dot in the plot gives a quick morphology overview of that cell.
 - ▶ Click the **Identify** button, in case adjustments for cell identification are necessary.
 - ▶ You can gate the data by using the **Define Region** button. Covered cells will be marked in a new color and further information on cell numbers and part of population (in %) can be seen in the **Regions** tab.
4. **Morphology data** can be viewed in a **histogram** where you can change the **cell feature** via the drop-down list. You can save the plot and alter the settings, grid lines and label viewing options, too.
5. Choose the **Export** tab to export data to **Excel** for further analysis. The exported data include information on the **image frames used, individual cell morphology parameters** and details on defined regions.
 - ▶ You can also **save** the analysis to return to it later.



Cell Morphology tab