# HOLOMONITOR® APP SUITE PROTOCOL

## WOUND HEALING ASSAY

This protocol helps to set up a Wound Healing Assay using HoloMonitor® M4 and the HoloMonitor® App Suite software. The **HoloMonitor® Wound Healing Assay** provides an automated label-free wound healing assay, measuring gap closure. Additionally, selected cells can be individual cell tracked for detailed individual cell movement and morphology analysis.

## REQUIREMENTS:

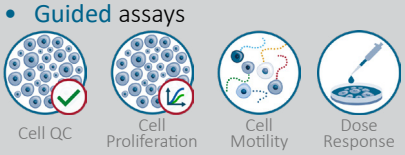
- HoloMonitor® M4, placed in incubator
- HoloMonitor® M4 App Suite
- Culture vessel of choice with cells
- HoloLid™ for selected vessel
- Vessel holder for selected vessel

## OUTPUT:

- Gap width (µm)
- Cell covered area (µm² and %)
- Cell free area (µm² and %)

## REANALYSIS:

- Guided assays



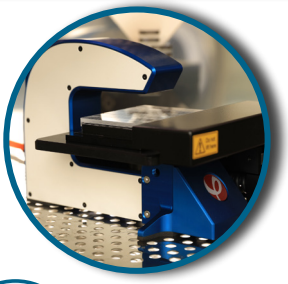

- In-depth assays


## 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. Perform In-Depth analysis
3. Export results
4. Create new results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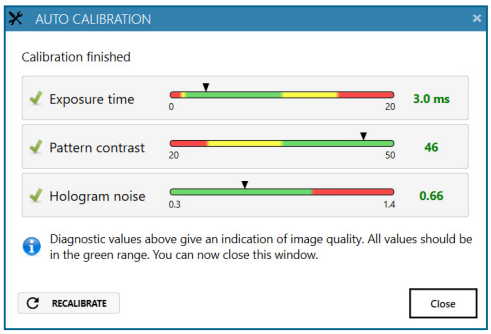
# PREPARATIONS

## Materials

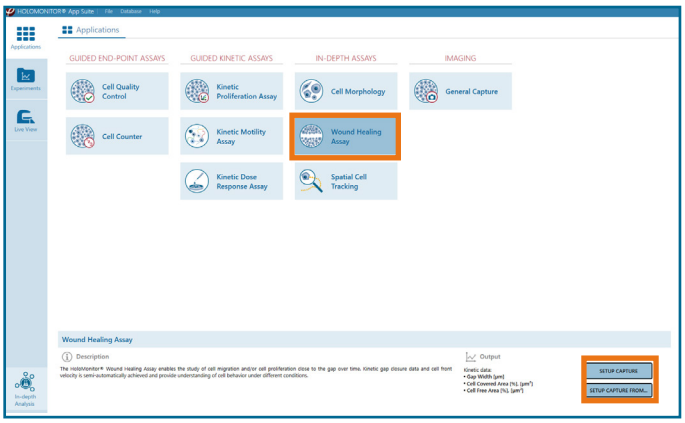
- ✓ **HoloMonitor® M4**, placed inside the incubator
- ✓ **HoloMonitor® App Suite** software
- ✓ **Cell culture vessel.**
  ► We recommend using ibidi® µ-Dish 35 mm with Culture-Insert 2 Well high (cat. # 81176) or ibidi® µ-Plate 24-well with Culture-Insert 2 Well (cat. # 80241)
- ✓ **HoloLid™** for the selected vessel
- ✓ **Vessel holder** for the selected vessel
- ✓ **Cells**
- ✓ **Setup and Operational Manual** for HoloMonitor ®M4

## Steps

1. 1Seed the cells to at least **90 % confluence** in the inserts according to ibidi protocol.
2. Place the vessel into 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. Remove the inserts and add respective cell **treatment**. The **final working volume** per well for recommended ibidi® vessels is 2.5 mL.
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 **Wound Healing Assay** and proceed by clicking the **Setup Assay** button.




Successful auto-calibration window

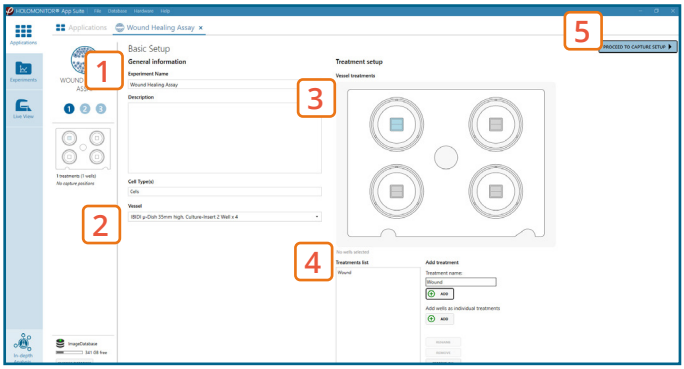



AppSuite main window with selected Wound Healing **Assay**

# 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**.

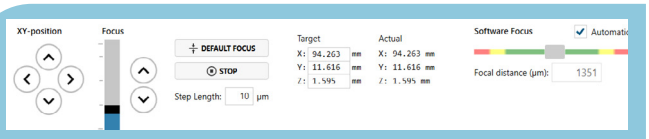



Basic Setup window

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

1. Adjust the default settings for **duration**, **interval**.
2. Add **capture positions**: The position list is open by default. Click positions on the vessel map and add them to the position list with the **Add current location button**. In case the image quality is poor, a warning sign ⚠ appears. **Adjust focus** or **position location** if necessary.
   ▶Note that the gap might not be exactly where the vessel map indicates.
3. Ensure that the **storage requirement** for the experiment does not exceed the computer capacity.
4. Run a full or quick **validation** of the selected positions to ensure **good image quality**.
5. When satisfied with the experiment setup, click **Proceed to Capture**.
   ▶If there are no position with both wound edges visible at the same time, add two positions which are parallel to each other and have one edge with cells. Use **XY position Arrow buttons** to move stage in steps and when satisfied, press **Add Current Location** button.


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

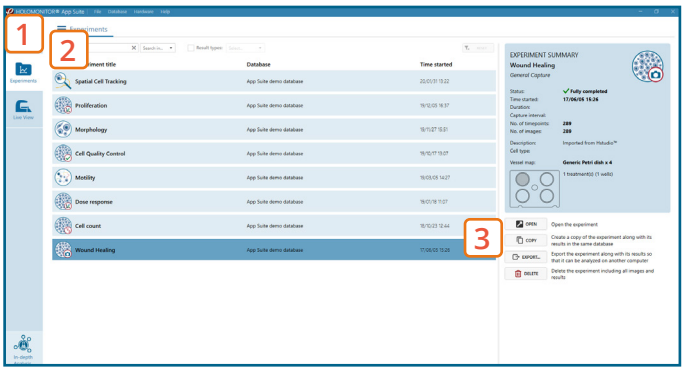
1. Click **Start Capture**.
2. To stop the experiment ahead of time, click the stop button. Note that it is NOT possible to restart the experiment once it has been stopped.
3. Go to the **Experiments tab** and open your ongoing experiment to preview the captured images during the run.
   ▶Wait for the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


Capture window

# RESULTS & ANALYSIS

## Experiments tab

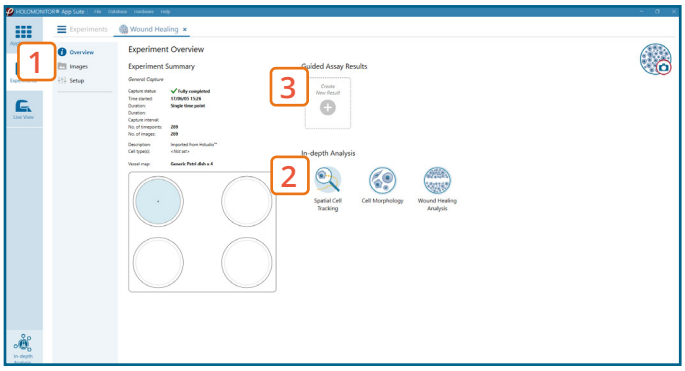
1. Click Experiments to see a **list of the experiments**.
2. Click on the experiment title to open an **experiment summary**.
3. Click **Open** to open the results page.




Experiments tab

## Experiment overview tab

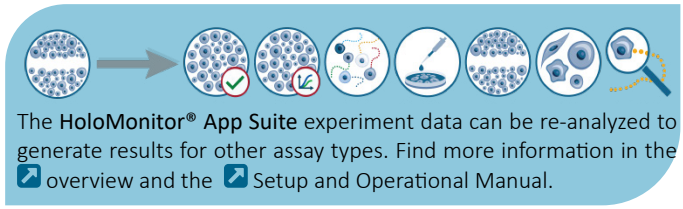
1. See the experiment **summary**, view all **images** and go to the experiment **setup** by choosing the respective tab.
2. Generate **in-depth analysis** data from the captured images by clicking on the **Wound Healing** icon.
   A **new window** for the in-depth analysis will open.
3. **Create New Guided Assay Results** from this experiment by clicking the respective button.



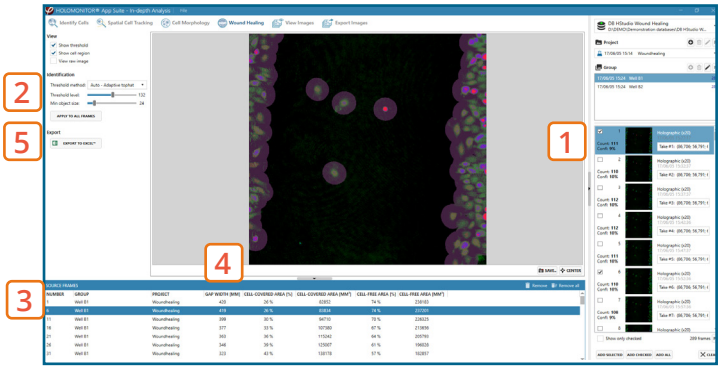

Experiment overview tab

## In-depth analysis — wound healing tab

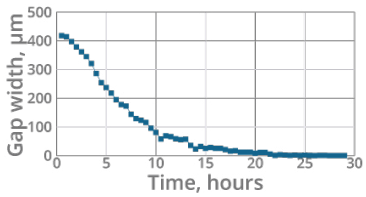
1. Begin by **adding frames** to the analysis either by drag-and-drop or using the Add selected or Add all button.
   ►Check the image quality before using it for in-depth analysis. See the **image guide** for more information.
2. **Adjust** the image **threshold and object size** and click **Apply to all frames**. You can alter the viewing options, too.
3. Based on the identification in the previous step, result values are generated and displayed in the **table**:
   ►gap with (mm), cell-covered area (% and µm²) and cell-free area (% and µm²).
4. **Check** the **gap width values** in the table to ensure that the values decrease evenly with time.
   ►In case of outliers, select the outlier frame in the list and **adjust the threshold** for that specific frame.
5. **Export** to **Excel** for further analysis. The **exported data** include: gap with (µm), cell-covered area (% and µm²) and cell-free area (% and µm²) and the settings overview.
   ►From the results it is easy create a **gap closure** graph or visualize the cell-covered area change over time and get the speed of the cell front (cell front velocity).
   ►If there was only one edge with cells visible in a field of view. To evaluate gap closure kinetics, calculate **cell front velocity** for each edge with cells and assess **cell covered area (%)** change in the field of view.



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.




Wound healing tab




Gap closure graph

Calculate the cell front velocity by dividing the first gap width value of the linear phase of the graph by the time value at the end of the slope's linear phase. In the example above: 410 µm/10.5 h= 39 µm/h