

HOLOMONITOR® APP SUITE PROTOCOL

WOUND HEALING

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:

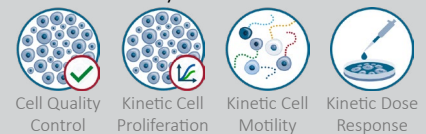
- **HoloMonitor® M4**, placed in incubator
- **HoloMonitor® M4 App Suite**
- **Culture vessel** of choice with **cells**
- **HoloLid™** for selected vessel
- **Vessel holder** for selected vessel

OUTPUT:

- **Gap width** (μm)
- **Cell covered area** (μm^2 and %)
- **Cell free area** (μm^2 and %)

REANALYSIS:

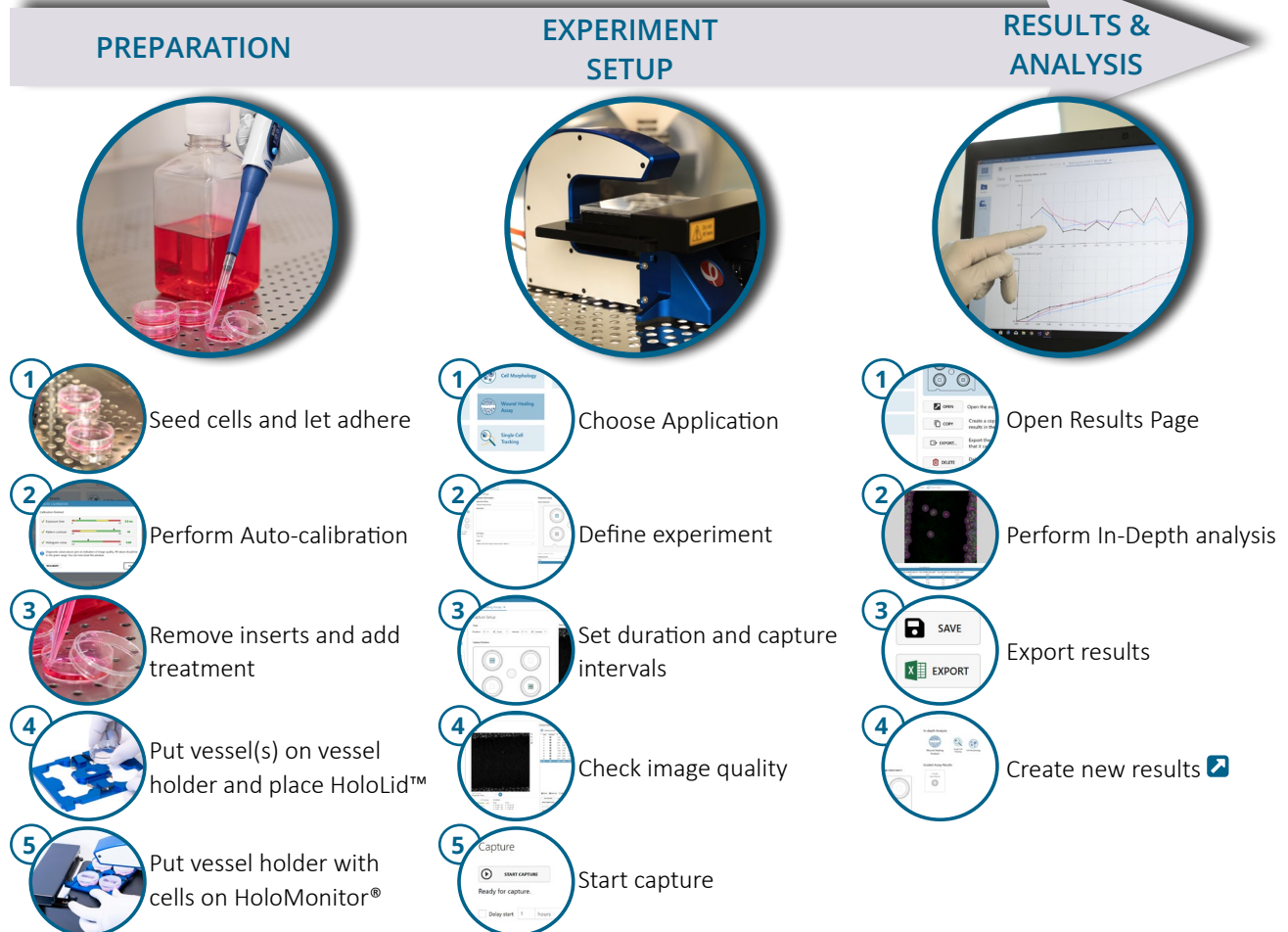
- **Guided assays**



- **In-depth assays**




WORK FLOW:





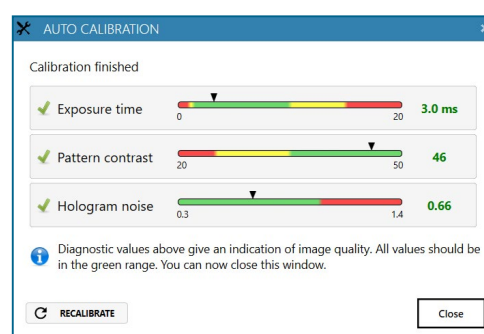
PREPARATIONS

Materials

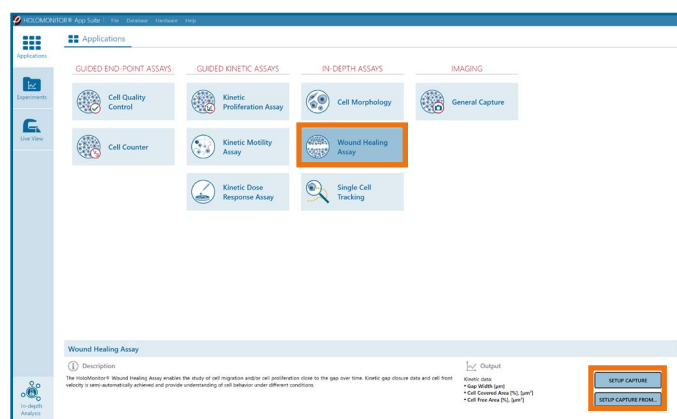
- ✓ HoloMonitor® M4, placed inside the incubator
- ✓ HoloMonitor® App Suite software
- ✓ Cell culture vessel.
 - We recommend using ibidi® μ -Dish 35 mm with Culture-Insert 2 Well high (cat. # 81176) or ibidi® μ -Plate 24-well with Culture-Insert 2 Well (cat. #80241)
- ✓ HoloLid™ for the selected vessel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

Steps

1. Seed 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  HoloLid™ sterilization and use 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**.
10. Select the **Wound Healing** application and proceed by clicking the **Setup Capture** button.



Successful auto-calibration window

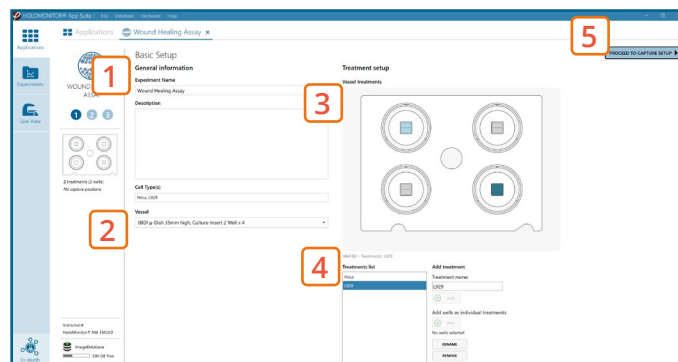


AppSuite main window with selected Wound Healing application

EXPERIMENT SETUP

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

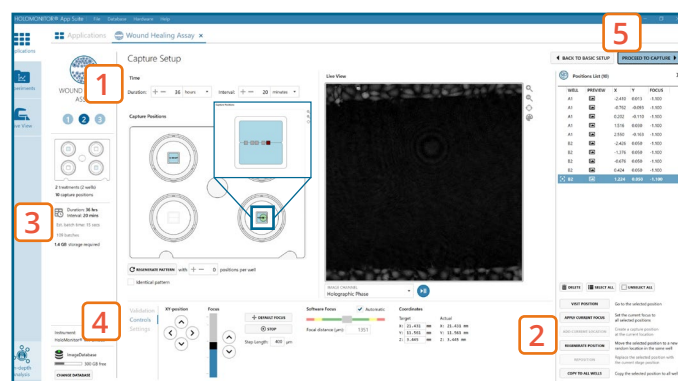
1. Enter the experiment **name**, optional experiment **description** and cell types.
2. Select the correct **vessel map** from the drop-down list.
3. Map **treatments and conditions** on the vessel map. **Select wells** by marking them with the left mouse button while moving the cursor over the well/s.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



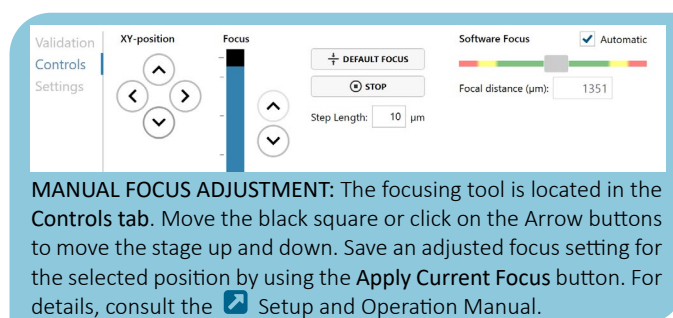
Basic Setup window

2 Capture setup: Select the experiment time settings, 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.
 - 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.
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.



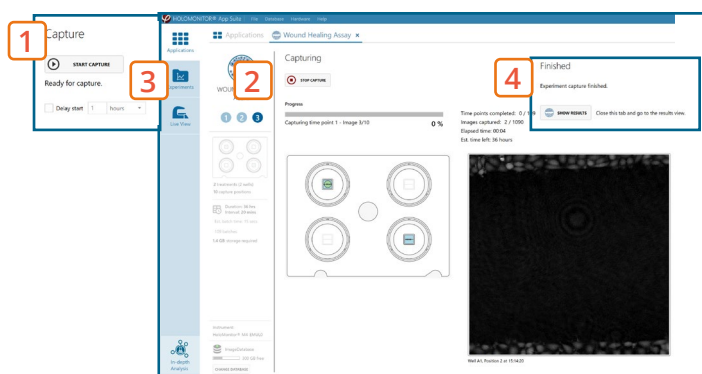
Capture Setup window



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

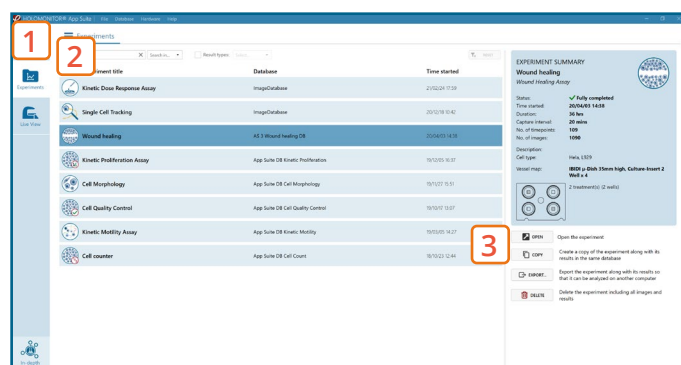


Capture window

RESULTS & ANALYSIS

Experiments tab

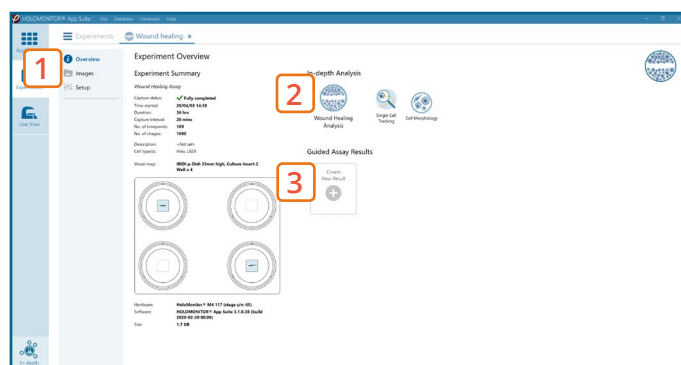
1. Click Experiments to see a list of the experiments.
2. Click on the experiment title to open an **experiment summary**.
3. Click **Open** to open the results page.



Experiments tab

Experiment overview tab

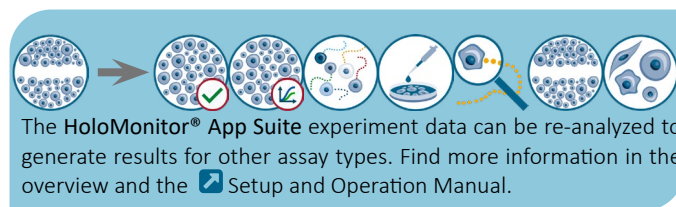
1. See the experiment **summary**, view all **images** and go to the experiment **setup** by choosing the respective tab.
2. Generate **in-depth analysis** data from the captured images by clicking on the **Wound Healing** icon. A **new window** for the in-depth analysis will open.
3. **Create New Guided 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 quality guide](#) for more information.
2. **Adjust the image threshold and object size** and click **Apply to all frames**. You can alter the viewing options.
3. Based on the identification in the previous step, result values are generated and displayed in the **table**:
► gap width (mm), cell-covered area (% and μm^2) and cell-free area (% and μm^2).
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.



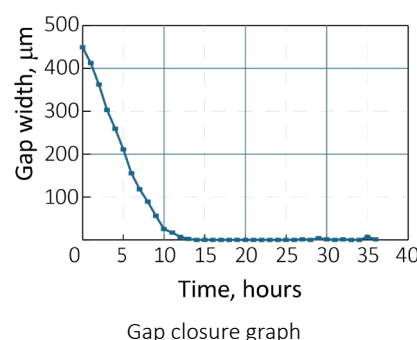
Wound healing tab

5. **Export to Excel** for further analysis. The **exported data** include: gap width (μm), cell-covered area (%) and μm^2) and cell-free area (%) and μm^2) 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.

It is the users responsibility to verify that the software algorithm was correctly applied during analysis and that results are accurate.




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: $410 \mu\text{m} / 10.5 \text{ h} = 39 \mu\text{m/h}$


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

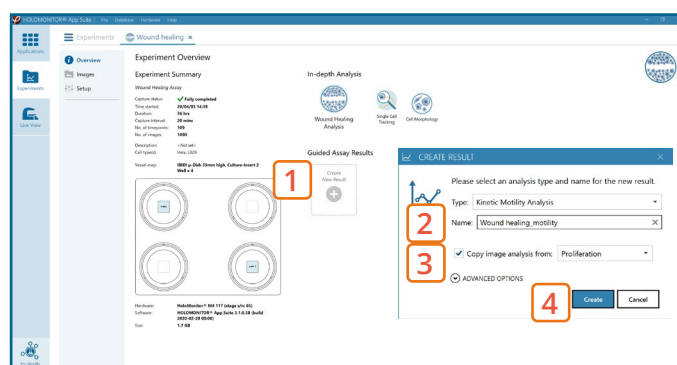
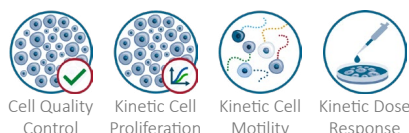
1. In the **Experiment overview** page under Guided Assay Results select **Create New Result**.
2. **Choose type** of analysis in the pop up window and name the new result.
3. Tick **copy image analysis from** and select the experiment to copy from. This will copy the image analysis settings from the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

Obtain these results from the Wound Healing data:

In-depth assays



Guided assays



Experiment overview tab

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