

HOLOMONITOR® APP SUITE PROTOCOL

KINETIC MOTILITY ASSAY

This protocol helps to set up **Kinetic Motility Assay** using **HoloMonitor® M4** and the **HoloMonitor® App Suite** software. The assay is designed to explore cell population motility over time. In the results tab you will obtain data on cell speed ($\mu\text{m}/\text{h}$), and accumulated mean distance (μm).

To optimize capture duration and capture frequency settings for your cell line, we recommend performing a 4 hours pre-study to determine the cell velocity, described in **Pre-Study Kinetic Motility** protocol. We recommend to perform each experiment at least three times to obtain sufficient data for statistical analysis.

REQUIREMENTS:

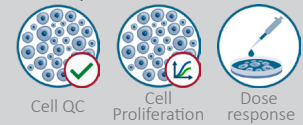
- HoloMonitor® M4, placed in incubator
- HoloMonitor® M4 App Suite
- Culture vessel of choice with cells
- HoloLid™ for selected vessel
- Vessel holder for selected vessel

OUTPUT:

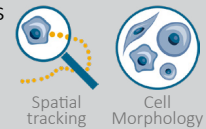
- Mean cell speed ($\mu\text{m}/\text{h}$)
- Accumulated mean cell distance (μm)

REANALYSIS:

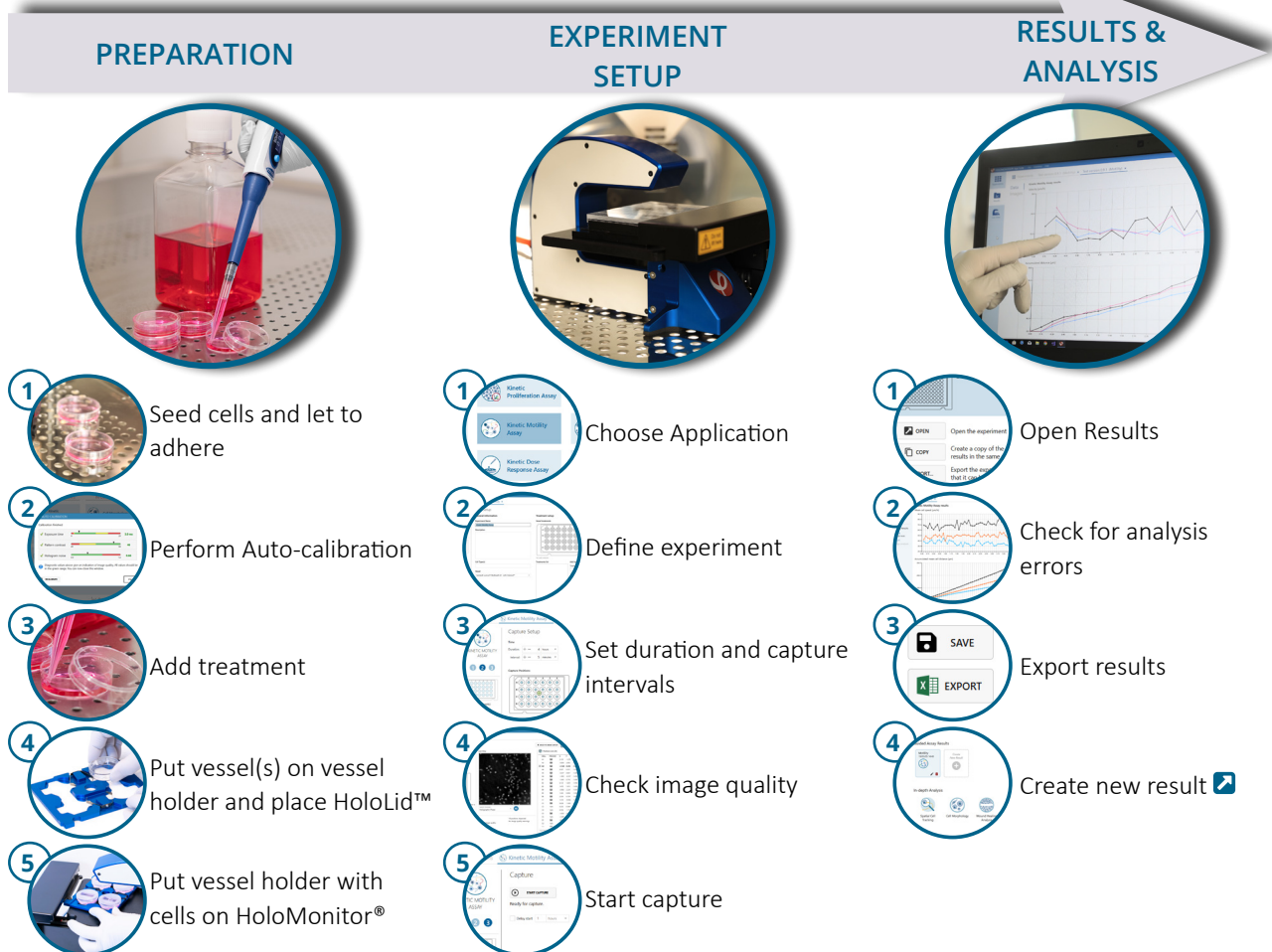
- Guided assays



- In-depth assays



WORK FLOW:



PREPARATIONS

Materials

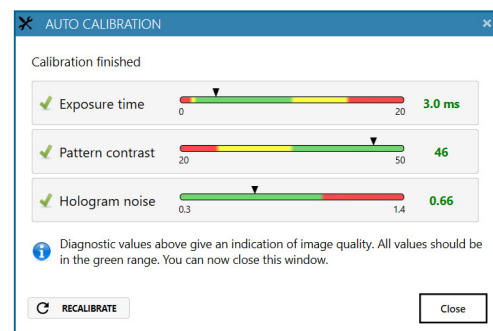
- ✓ HoloMonitor® M4, placed inside the incubator
- ✓ HoloMonitor® App Suite software
- ✓ Cell culture vessel. Please check our [list](#) with recommended vessels.
- ✓ HoloLid™ for the selected vessel
- ✓ Vessel holder for the selected vessel
- ✓ Cells
- ✓ [Setup and Operational Manual](#) for HoloMonitor®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

Steps

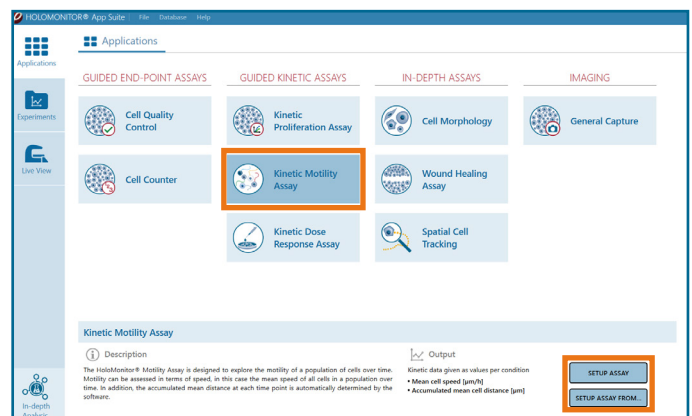
1. Seed the cells with about 5 % confluence (ca. 6000 – 11000 cells/cm²).
 - ▶ Please note that too few cells may lead to inadequate results due to auto-focus failure.
2. Place the vessel into the incubator and let cells attach for 2-24 hours.
3. Start the software and wait for complete instrument initialization.
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. 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 Kinetic Motility Assay and proceed by clicking the Setup Assay button.



Successful auto-calibration window

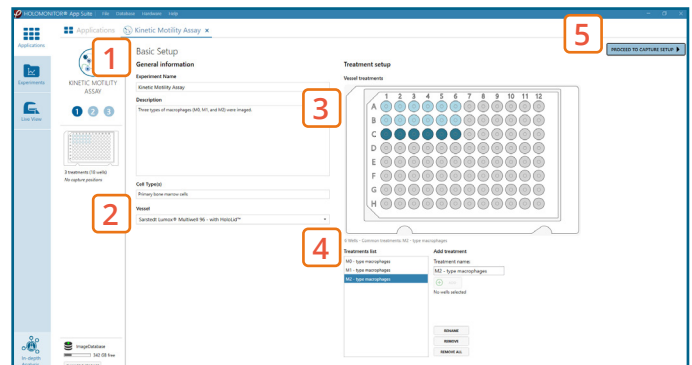


AppSuite main window with selected Kinetic Motility Assay

EXPERIMENT SETUP

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

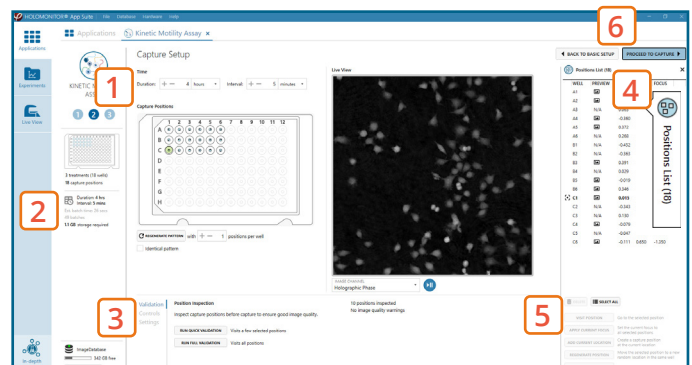
1. Enter the experiment name, optional experiment description and cell types.
2. Select the correct vessel map from the drop-down list.
3. Map treatments and conditions on the vessel map. Select wells by marking them with the left mouse button while moving the cursor over the relevant well/s.
4. Add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



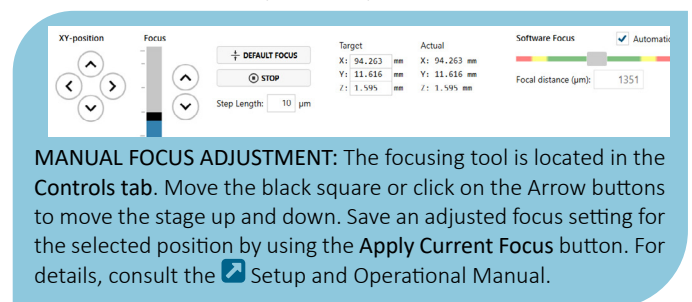
Basic Setup window

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



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 results during the run.
4. When the Experiment capture finishes, click the Show Result button to get directly to the Results page.

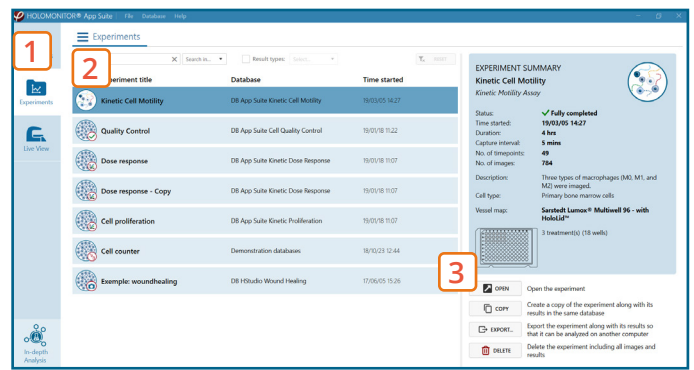


Capture window

RESULTS & ANALYSIS

Experiments tab

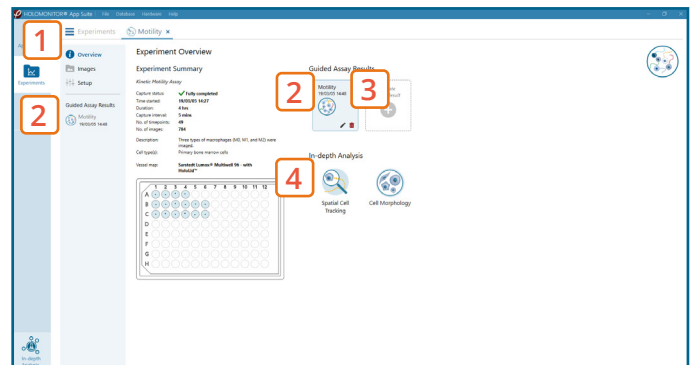
1. Click Experiments to see a list of the experiments.
2. Click on the experiment title to open an experiment summary.
3. Click **Open** to open the results page.



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. Open the **experiment data** by clicking on the experiment result icon or its name in the Guided Assay Result list.
3. **Create New Results** from this experiment by clicking the button.
4. Reach **In-depth Analysis** by clicking the respective icon.



Experiment overview tab

Kinetic motility data results tab

- ✓ The assay results are generated during image capturing.
- ✓ The software automatically analyzes quantitative data generated from each image.
- ✓ For a more detailed analysis all results can be exported.

1. The results are given as graphs showing **Mean cell speed** ($\mu\text{m}/\text{h}$) and **Accumulated mean cell distance** (μm).
2. **Enlarge the graphs** to have a closer look.
3. For further analysis, **export** all results to Excel. The exported data include **population data based on means**, including data for each captured position and standard deviations.

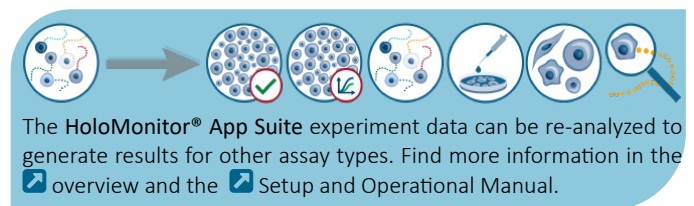
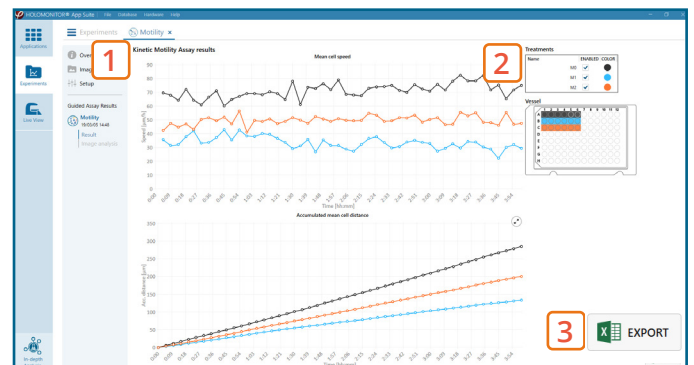


Image analysis tab

- ✓ An image analysis algorithm filters out images of poor quality and excludes them from results data.
- ✓ Include or exclude images from results analysis. See the [image guide](#) for more information.



Motility results tab

1. The captured images from the experiment are displayed in a **tree-view list**. Clicking an item in the tree will show a list of all images contained in the selected level/category.
2. **Checked check boxes** — all images are included. **Empty check boxes** — all images are excluded. **Check boxes with a dash** — some images are included. Symbols in the list show cell analysis or image quality warning.
3. It is possible to adjust the Sensitivity of image(s) and view the image threshold and markers.

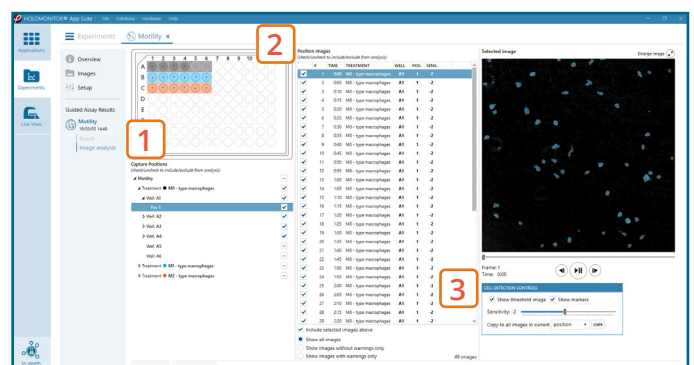


Image analysis tab