

# 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 ( $\mu\text{m}$ ).

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:

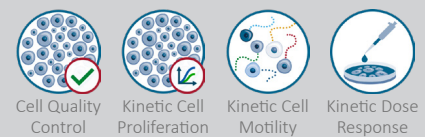
- HoloMonitor® M4, placed in incubator
- HoloMonitor® M4 App Suite
- Culture vessel of choice with cells
- HoloLid™ for selected vessel
- Vessel holder for selected vessel

### OUTPUT:

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

### REANALYSIS:

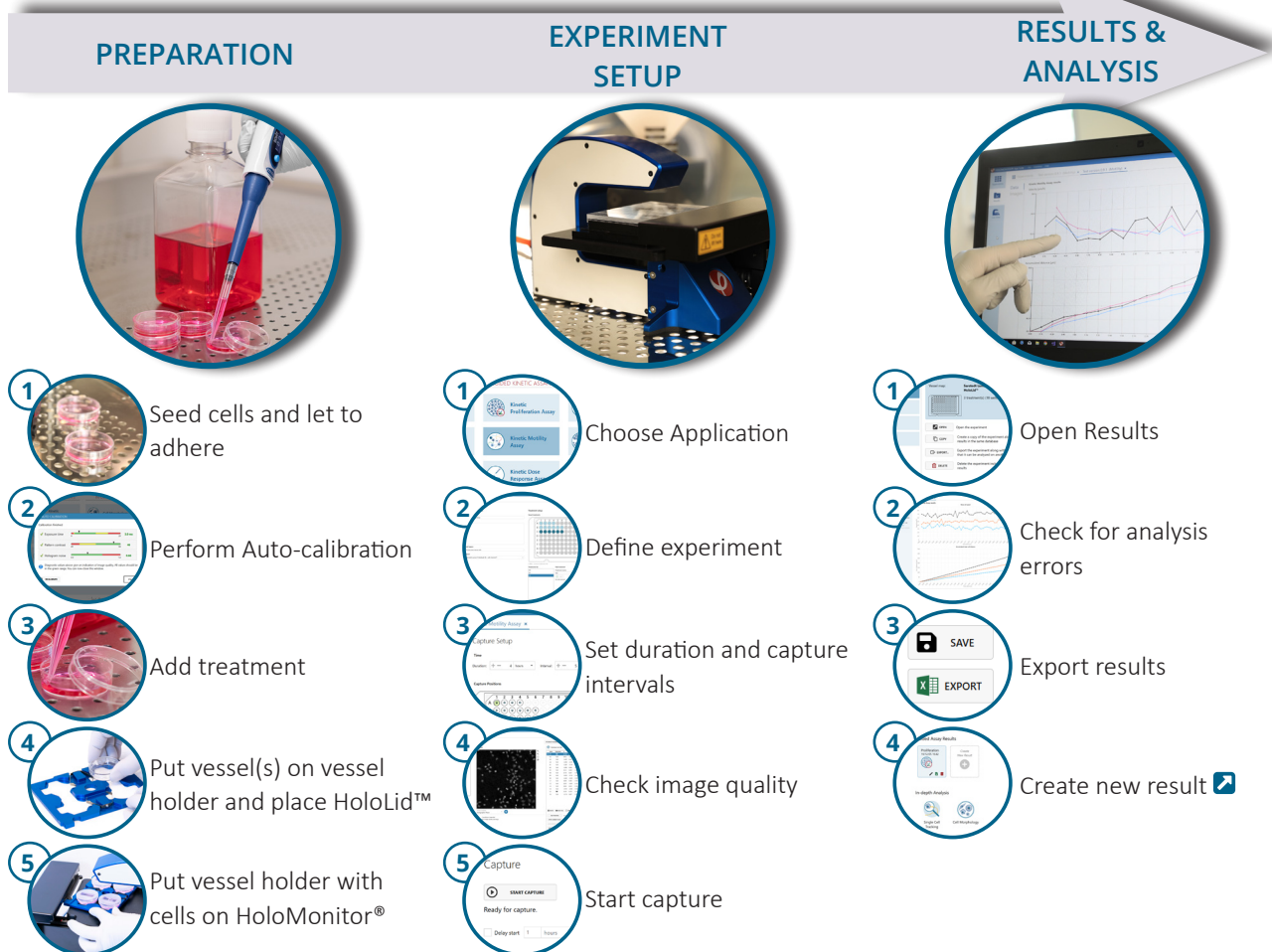
- Guided assays



- In-depth assays



### WORK FLOW:



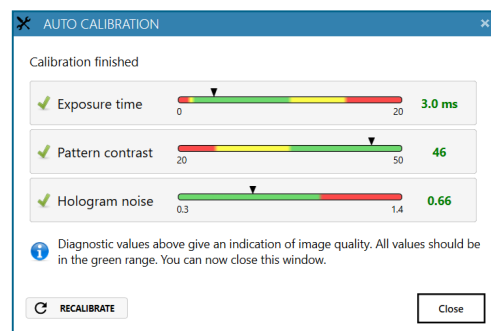
# PREPARATIONS

## 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 Operation Manual](#) for HoloMonitor® M4

## Steps

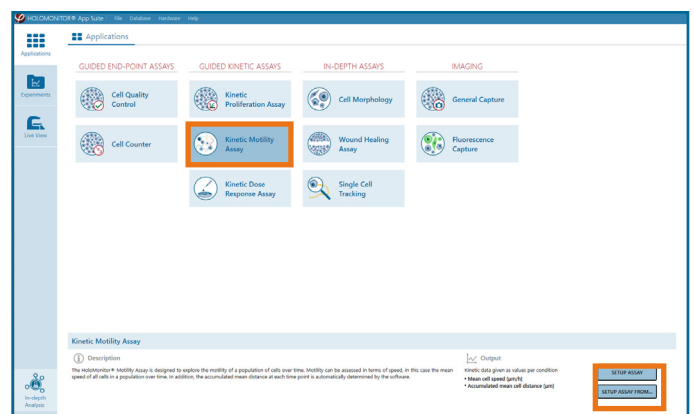
1. Seed the cells with about 5 % confluence (ca. 6000 – 11000 cells/cm<sup>2</sup>).
  - ▶ Please note that too few cells may lead to inadequate results due to auto-focus failure.
2. Place the vessel into the incubator and let cells attach for 2-24 hours.
3. Start the software and wait for complete instrument initialization.
4. Run an auto-calibration. With successful calibration, the instrument is ready to use.
5. Sterilize the HoloLids™ according to the [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, cm <sup>2</sup> /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 Kinetic Motility Assay and proceed by clicking the Setup Assay button.

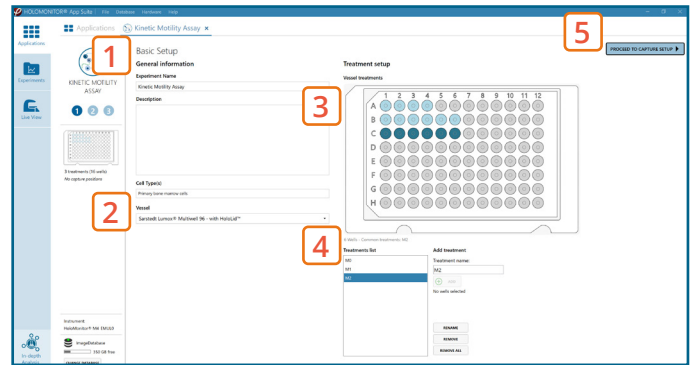


AppSuite main window with selected Kinetic Motility Assay

# EXPERIMENT SETUP

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

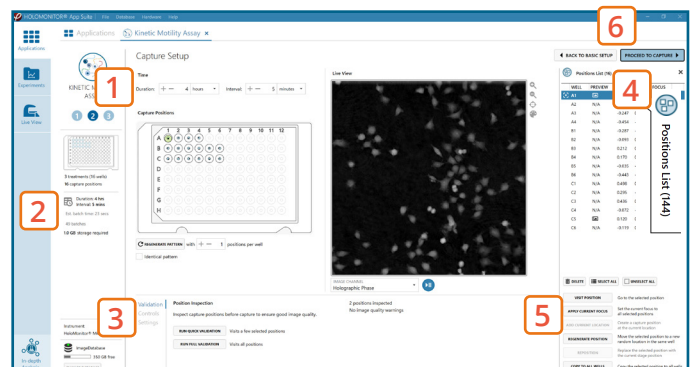
1. Enter the experiment name, optional experiment description and cell types.
2. Select the correct vessel map from the drop-down list.
3. Map treatments and conditions on the vessel map. Select wells by marking them with the left mouse button while moving the cursor over the relevant well/s.
4. Add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

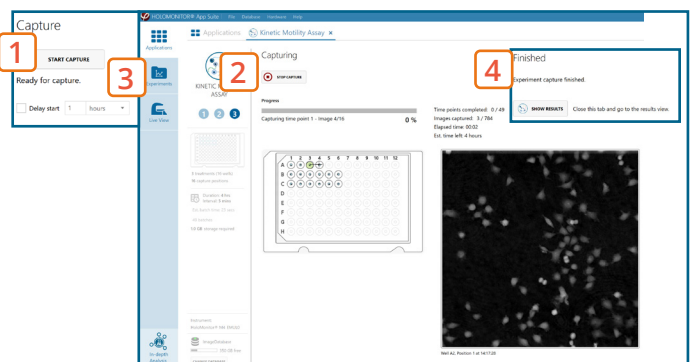


Capture Setup window

**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. 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.

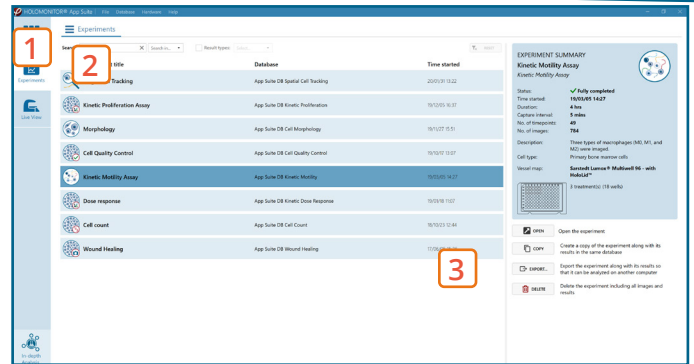


Capture window

# RESULTS & ANALYSIS

## Experiments tab

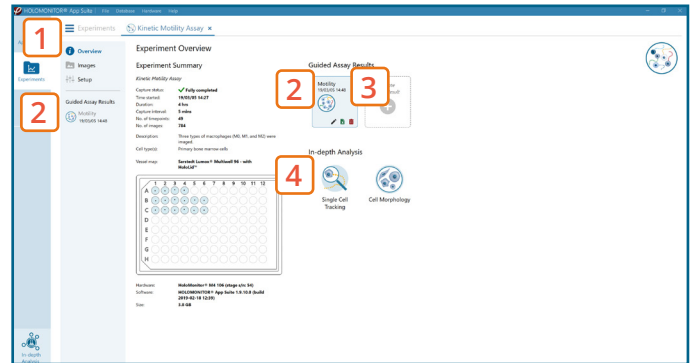
1. Click Experiments to see a list of the experiments.
2. Click on the experiment title to open an experiment summary.
3. Click **Open** to open the results page.



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.

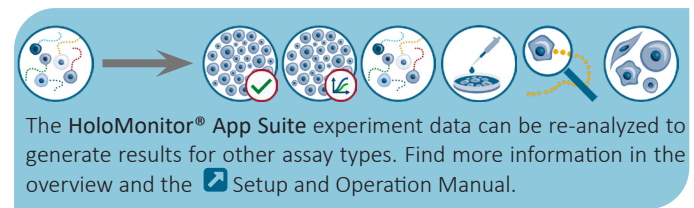


Experiment overview tab

## 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.

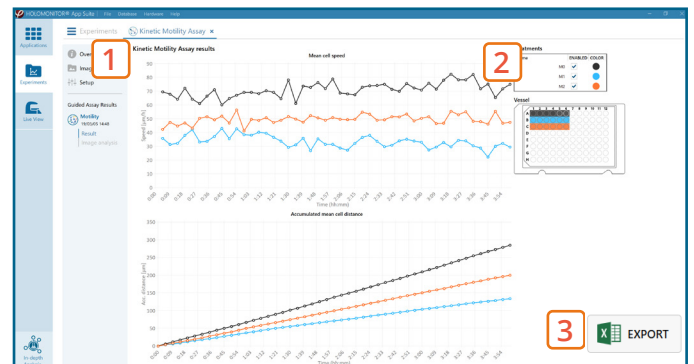
1. The results are given as graphs showing **Mean cell speed** ( $\mu\text{m}/\text{h}$ ) and **Accumulated mean cell distance** ( $\mu\text{m}$ ).
2. **Enlarge the graphs** to have a closer look.
3. For further analysis, **export** all results to Excel. The exported data include **population data based 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 quality guide](#) for more information.

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.



Motility results tab

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

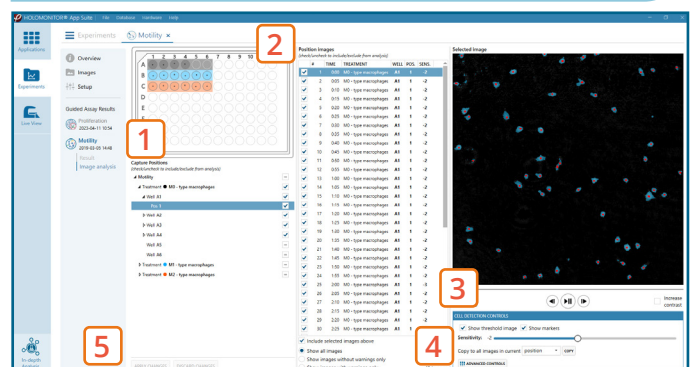


Image analysis tab

- The **Advanced controls** let the user adjust **Threshold value**, **Threshold method** and **Min. cell size** so the segmentation mask fits the holography signal. Choose which frames to apply the changes to in the dropdown menu and click **Copy** to apply the changes.
  - ▶ Check cell segmentation for some images in the beginning, middle, and end of the experiment to make sure the applied threshold fits.
- Once satisfied with the cell identification, click **Apply changes** to save the changes.

## One experiment — multiple results

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

### Generating Guided Assay results

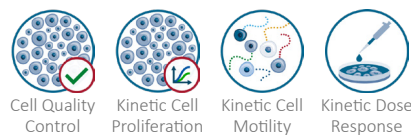
- In the **Experiment overview page** under Guided Assay Results select **Create New Result**.
- Choose type** of analysis in the pop up window and name the new result.
- Tick **copy image analysis from** and select the experiment to copy from. This will copy the image analysis settings from the selected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

### Generating In-depth Assay results

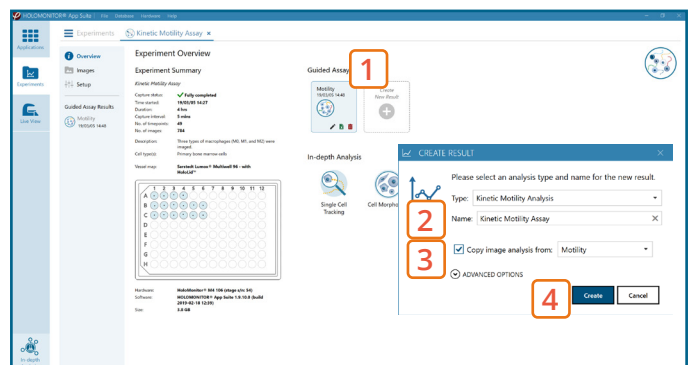
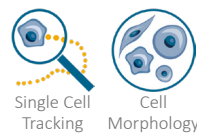
- In the **Experiment overview page** select the In-depth application icon for wanted result.
- Follow** the respective assay protocol [↗](#).

Obtain these results from the Kinetic Motility data:

**Guided** assays



**In-depth** assays



Experiment overview tab