

# 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**



Cell Quality Control



Kinetic Cell Proliferation



Kinetic Cell Motility



Kinetic Dose Response

- **In-depth assays**

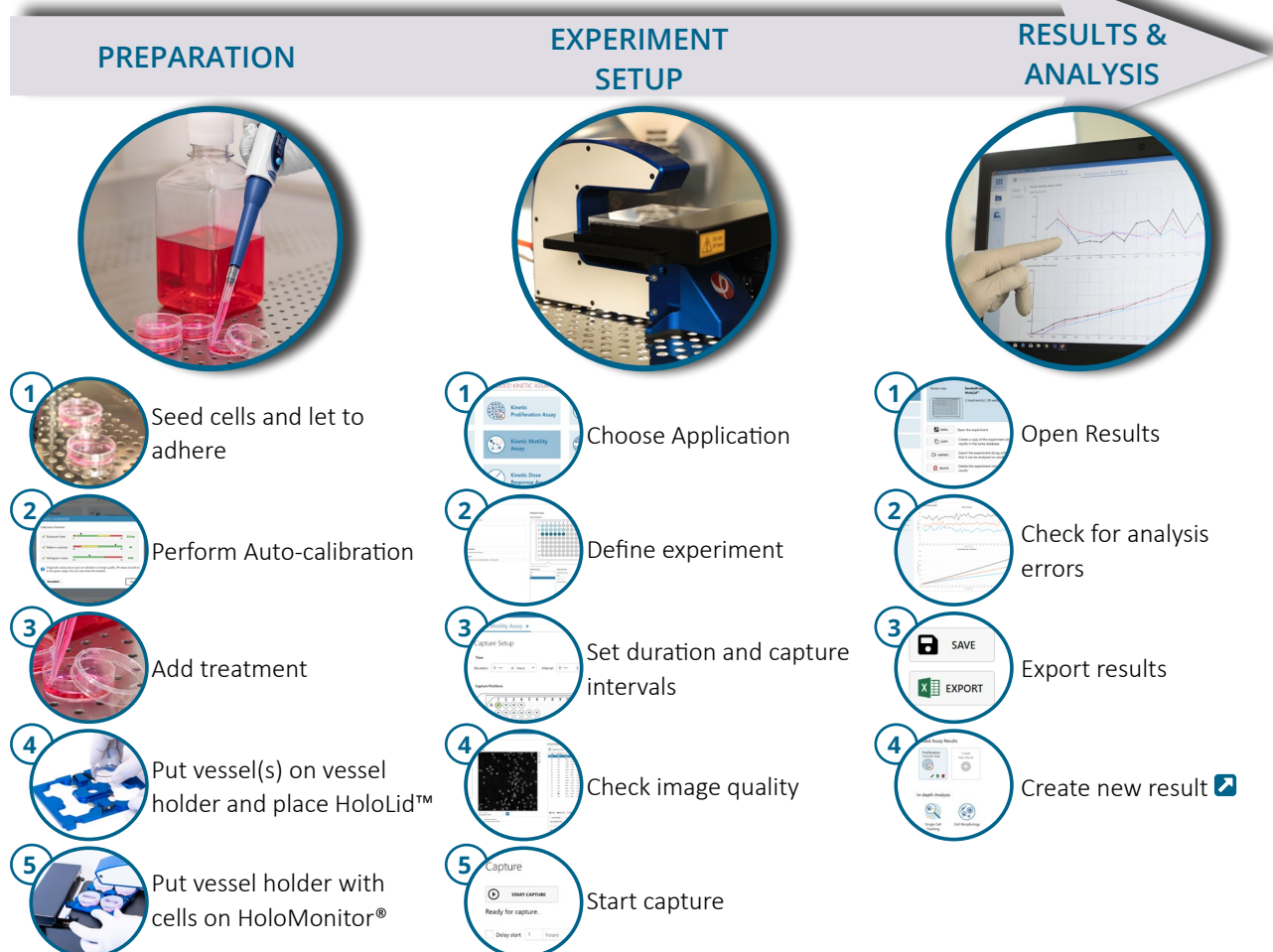


Single Cell Tracking



Cell Morphology

### WORK FLOW:



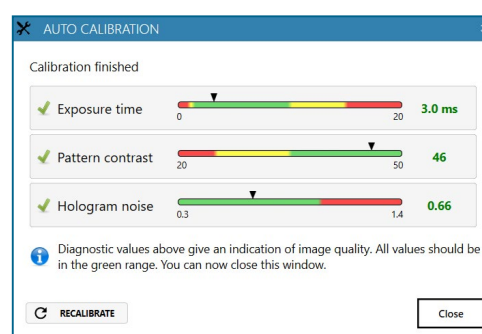
## PREPARATIONS

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

### Steps

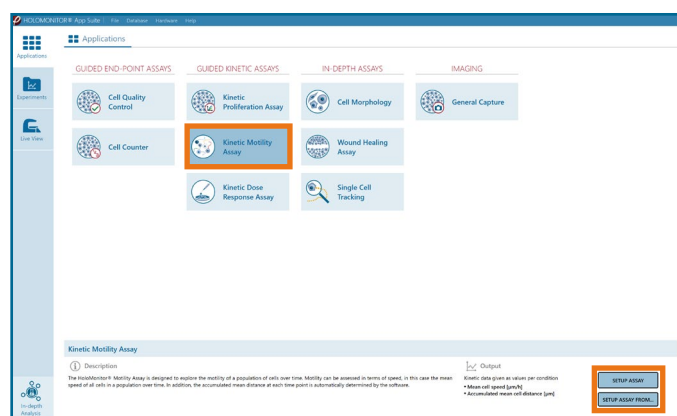
1. Seed the cells with about **5 % confluence** (ca. 6000 – 11000 cells/cm<sup>2</sup>).  
► Please note that too few cells may lead to inadequate results due to auto-focus failure.
2. Place the vessel into the incubator and let **cells attach** for 2-24 hours.
3. **Start the software** and wait for 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.

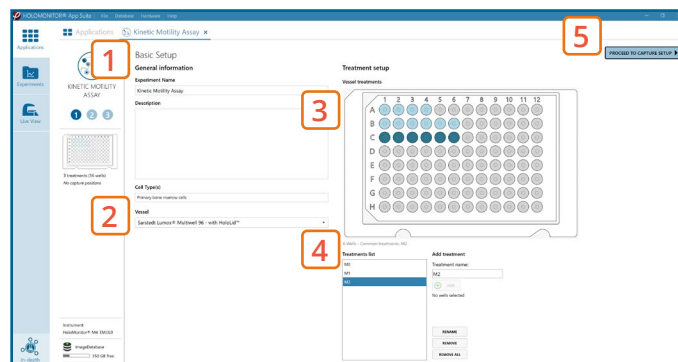


AppSuite main window with selected Kinetic Motility Assay

## EXPERIMENT SETUP

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

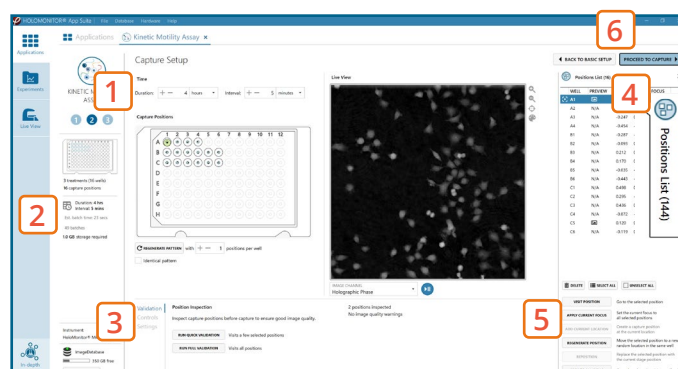
1. Enter the experiment **name**, optional experiment **description** and cell types.
2. Select the correct **vessel map** from the drop-down list.
3. Map **treatments and conditions** on the vessel map. **Select wells** by marking them with the left mouse button while moving the cursor over the relevant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



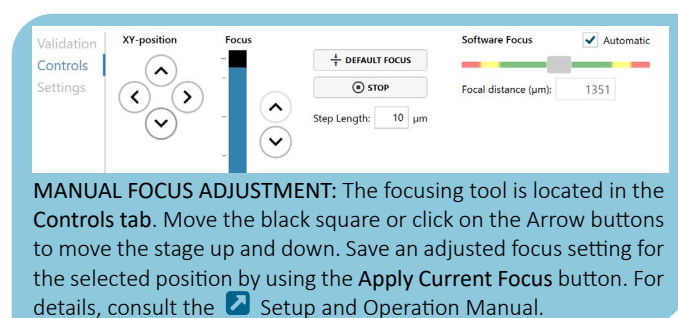
Basic Setup window

### 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**.

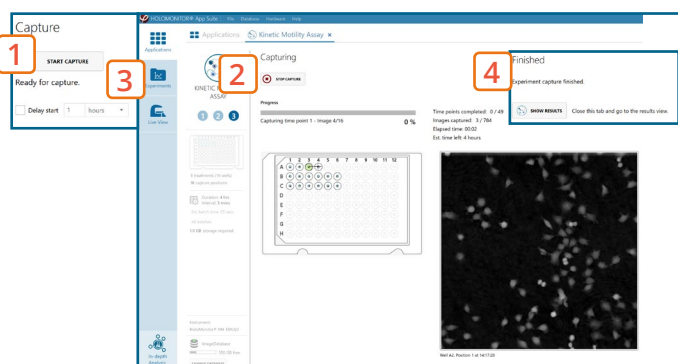


Capture Setup window



### 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.

### 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.



### Kinetic motility data results tab

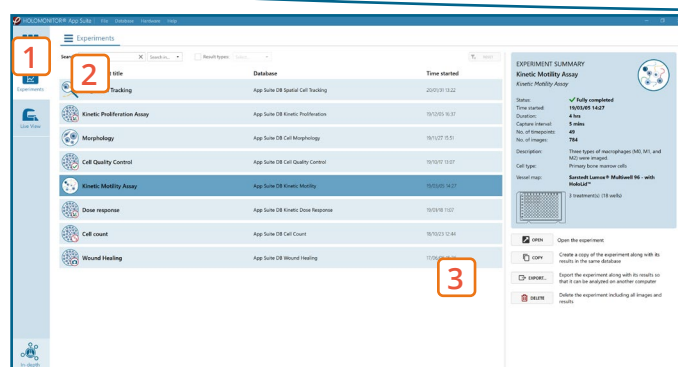
- ✓ The assay results are generated during image capturing.
- ✓ The software automatically analyzes quantitative data generated from each image.
- ✓ For a more detailed analysis all results can be exported.

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**.

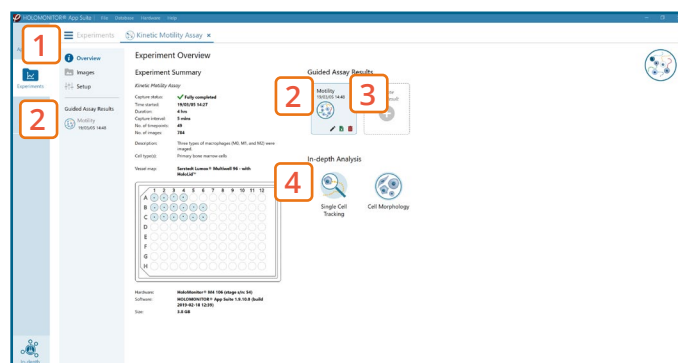
### Image analysis tab

- ✓ 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.

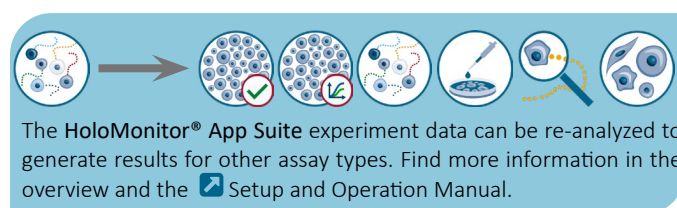
1. The captured images from the experiment are displayed in a **tree-view** list. Clicking an item in the tree will show a list of all images contained in the selected level/category.
2. **Checked check boxes** — all images are included. **Empty check boxes** — all images are excluded. **Check boxes 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.



Experiments tab



Experiment overview tab



Motility results tab

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

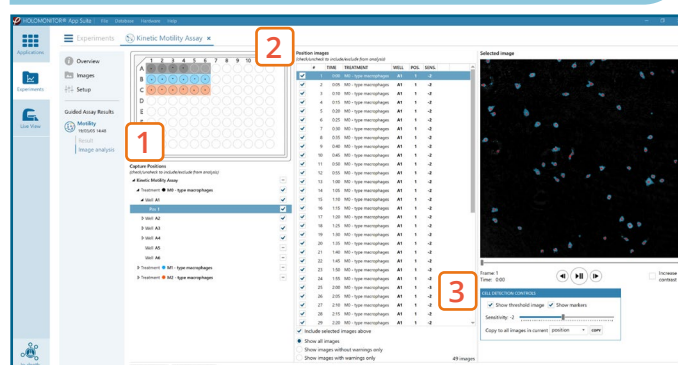


Image analysis tab



## One experiment — multiple results

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

### Generating Guided Assay results

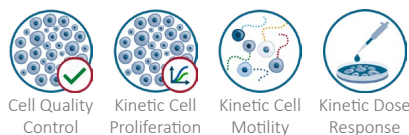
1. In the **Experiment overview** page under Guided Assay Results select **Create New Result**.
2. **Choose type** of analysis in the pop up window and **name** the new result.
3. Tick **copy image analysis from** and select the experiment to copy from. This will copy the image analysis settings from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

### Generating In-depth Assay results

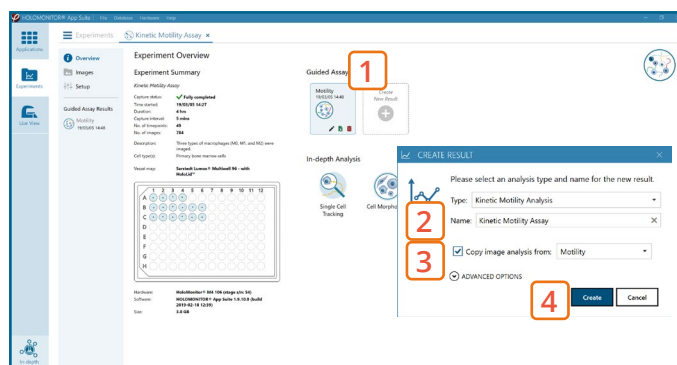
1. In the **Experiment overview** page select the In-depth application icon for wanted result.
2. **Follow** the respective assay protocol [↗](#).

Obtain these results from the Kinetic Motility data:

#### Guided assays



#### In-depth assays



Experiment overview tab