HOLOMONITOR® APP SUITE PROTOCOL
CELL MORPHOLOGY

This protocol helps to set up a Cell Morphology Assay using HoloMonitor® M4 and the HoloMonitor® App Suite software. The HoloMonitor® Cell Morphology Assay enables non-invasive live-cell quantification of a wide range of morphological properties, including individual cell volume, area and thickness.
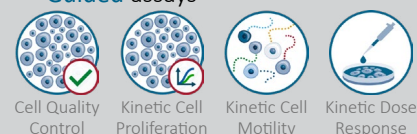
REQUIREMENTS:

- 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:

- 30+ features
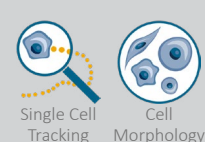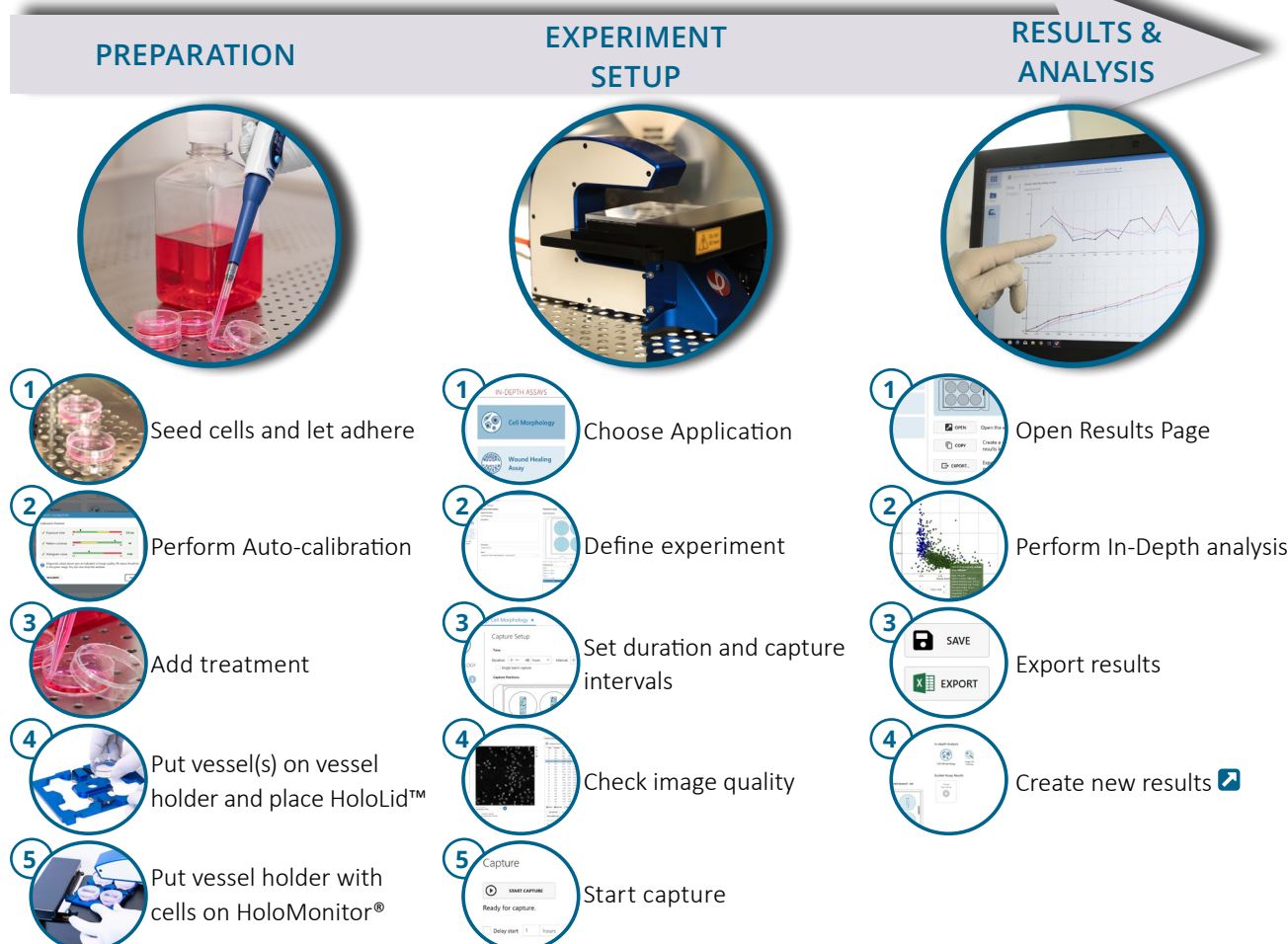- Calculation spread sheets of all data, including population averages

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:

PREPARATION

1. Seed cells and let adhere
2. Perform Auto-calibration
3. 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

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^2).
   ►Please note that too few cells may lead to inadequate results due to auto-focus failure.

2. Place the vessel in 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:
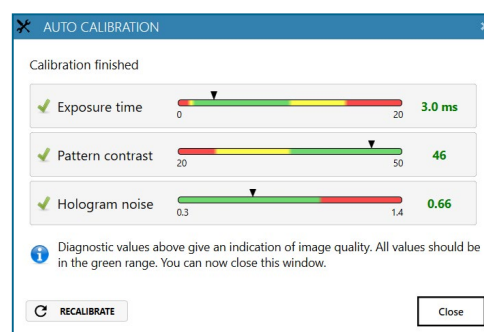
Successful auto-calibration window

Vessel	Vendor cat. number	HoloLid™	Final working volume	Growth area, cm²/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 **Cell Morphology** application and proceed by clicking the Setup Capture button.
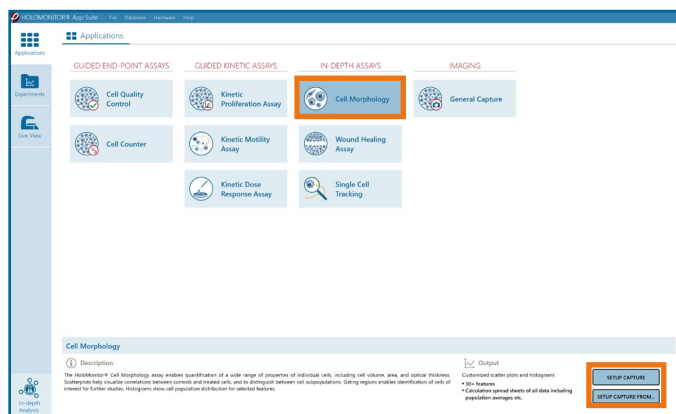
AppSuite main window with selected **Cell Morphology** application

EXPERIMENT SETUP

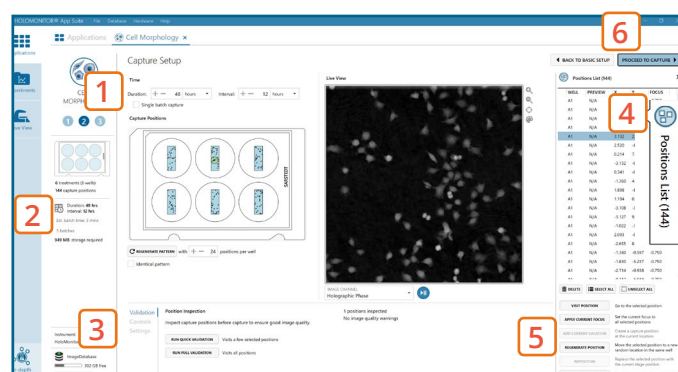## ① Basic setup: describe the experiment and assign treatments to the wells

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 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.
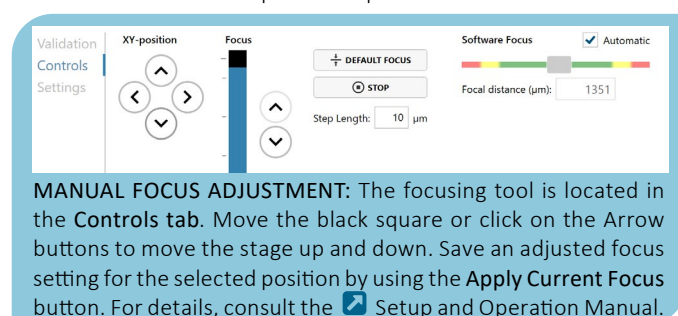5. Proceed to **Capture setup**.
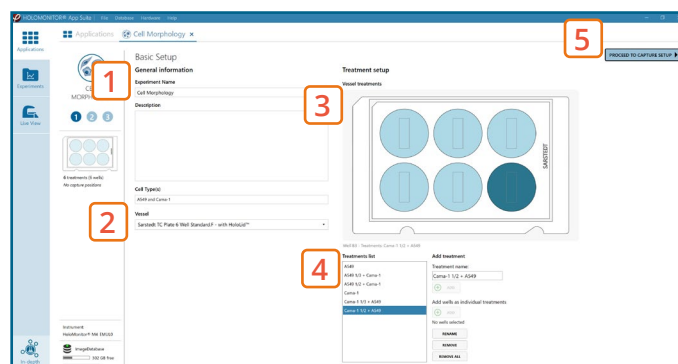
Basic Setup window

② Capture setup: Select the experiment time settings 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.
   ► 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.
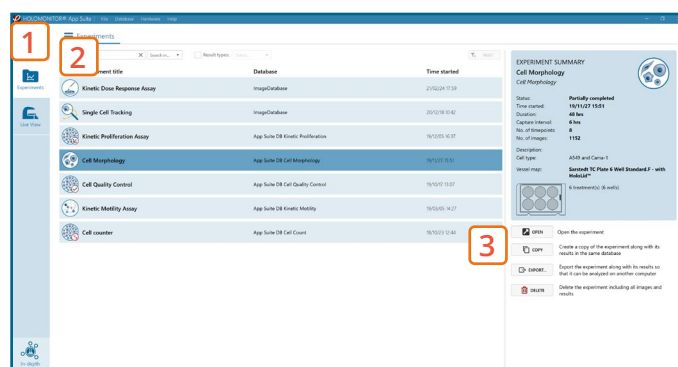4. When the Experiment capture finishes, click the **Show Result** button to get directly to the **Results page**.

Capture window

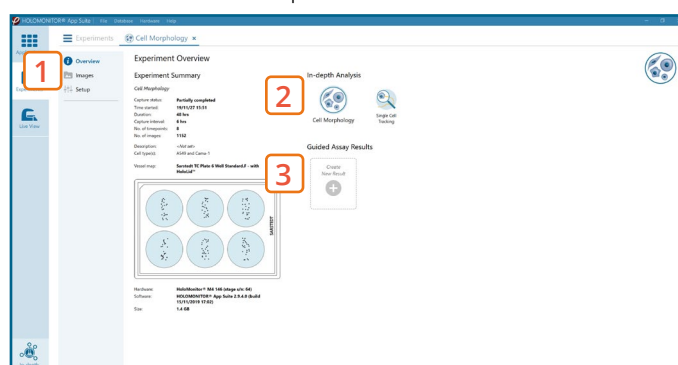# RESULTS & ANALYSIS

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. Generate **in-depth analysis** data from the captured images by clicking on the **Cell Morphology** 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

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 Operation Manual.

In-depth analysis — identify cells tab

✓ First step for morphology analysis is to identify the cells.

✓ The software automatically calculates cell count and confluence of each image.

✓ Review the image quality and include/ exclude images from results analysis. See the ⬈ **Image quality guide** for more information.

1. Start by selecting a **frame of interest**, so the image is displayed. You can alter the viewing options.

2. **Adjust** the background **threshold** and **object size** so the mask best fits the cell segmentation and click **Apply** (Current/ Checked/ All) to fix the settings to the frames and to display the **automatic calculation** of **cell count (#/frame)** and **confluence (%)**.

3. **Manual changes** are possible. Add/remove or grow/ shrink a cell, delete a cell area or draw a cell area yourself. These changes will **only** be saved to the **currently selected image**.

4. **Check** cell segmentation for some images in the beginning, middle and end of the experiment to make sure the applied threshold fits. Scrolling through the image list and checking for abnormal jumps in cell count or confluence values may help as well to see if adjustments are necessary.

5. Once satisfied with the cell identification, **click** on the **Cell Morphology** tab to begin analysis.
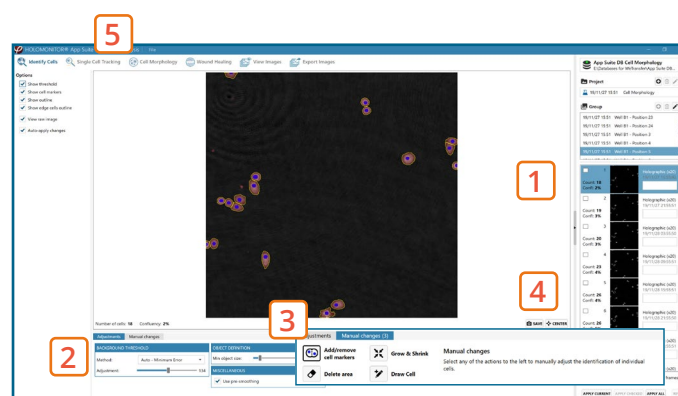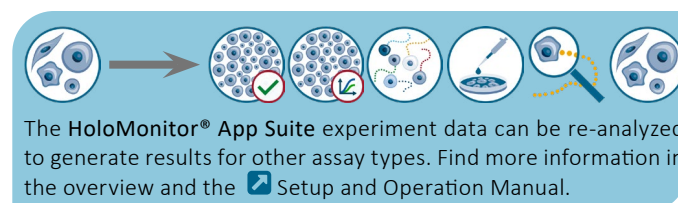

Identify cells tab

It is **important for in-depth analysis that cell identification is done correctly**. Thus, the more thoroughly the segmentation is performed, the better analysis results are in the following steps.

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

In-depth analysis — cell morphology tab

✓ Review the image quality and include/ exclude images from results analysis. See the 🡥 **Image quality guide** for more information.

✓ For further analysis all results can be exported.

✓ For more details, see the 🡥 **Setup and Operation manual**.
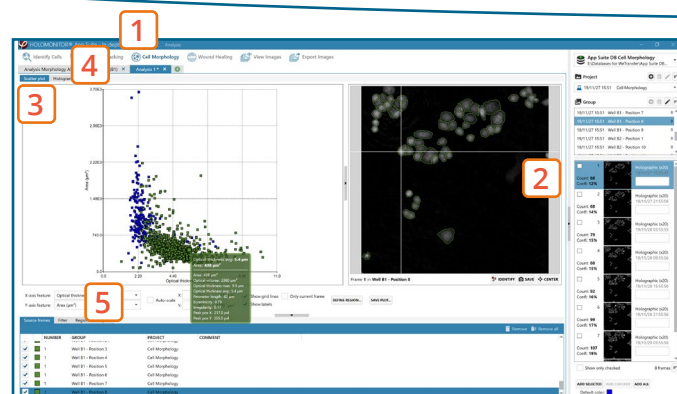
Cell Morphology tab

1. Click **New Analysis** to start a new data analysis or load a previous one by clicking the Open button.

2. **Add one** or **several frames** from the list to the analysis either by drag-and-drop or using the Add Selected/ Checked/ All button. You can sort the frames in the list by time or position.

3. **Morphology data** from all added frames is presented in a **scatter plot** where you can change the parameters of the x- and y-axis via the drop-down list. You can save the plot and alter scale, grid lines and label viewing options, too.
   ►The selected source frame is shown in the **image**. Hovering over a marked cell or a dot in the plot gives a quick morphology overview of that cell.
   ►Click the **Identify** button, in case adjustments for cell identification are necessary.
   ►You can gate the data by using the **Define Region** button. Covered cells will be marked in a new color and further information on cell numbers and part of population (in %) can be seen in the **Regions tab**.

4. **Morphology data** can be viewed in a **histogram** where you can change the **cell feature** via the drop-down list. You can save the plot and alter the settings, grid lines and label viewing options, too.

5. Choose the **Export tab** to export data to **Excel** for further analysis. The exported data include information on the **image frames used, individual cell morphology parameters** and details on **defined regions**.
   ►You can also **save** the analysis to return to it later.
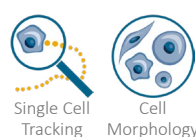
One experiment — multiple results

✓ This section helps to reanalyse data between different applications using HoloMonitor® App Suite software.
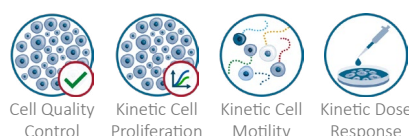
Generating In-depth Assay results

1. In the **Experiment overview page** select the In-depth application icon for wanted result.

2. **Follow** the respective assay protocol 🡥.

Obtain these results from the Cell Morphology data:

In-depth assays

Single Cell Tracking   Cell Morphology

Guided assays

Cell Quality Control   Kinetic Cell Proliferation   Kinetic Cell Motility   Kinetic Dose Response

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**.

Experiment overview tab

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