HOLOMONITOR® APP SUITE PROTOCOL
CELL QUALITY CONTROL ASSAY

This protocol helps to set up a Cell Quality Control Assay using HoloMonitor® M4 and the HoloMonitor® App Suite software. The HoloMonitor® Cell Quality Control assay can be used to ensure that the cell count and/or confluence is appropriate at start of an experiment. The Cell Quality Control Assay can also be used as a tool to detect undesired changes in your cell culture as compared to previous experiment(s).

REQUIREMENTS:

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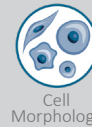- Vessel holder for selected vessel

OUTPUT:

- Cell count (cells/cm^2)
- Mean cell area (μm^2)
- Mean cell diameter (μm)
- Mean cell volume (μm^3)
- Confluence (%)

REANALYSIS:

- Guided assays

  Cell QC

- In-depth assays

  Cell Morphology

WORK FLOW:

PREPARATION	EXPERIMENT SETUP	RESULTS & ANALYSIS

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 number of positions
4. Check image quality
5. Start capture

RESULTS & ANALYSIS

1. Open Results
2. Check for analysis errors
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 Operational Manual** for HoloMonitor ®M4

Steps

1. Seed the cells with about **10 % confluence** (ca. 12000 – 22000 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 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:
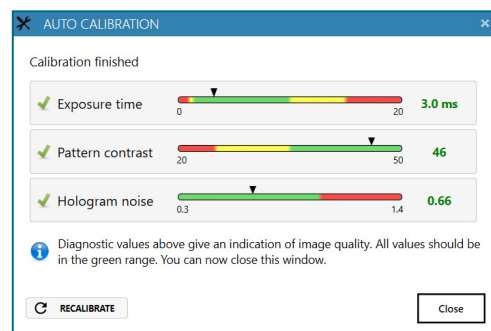
Successful auto-calibration window

Vessel	HoloLid™	Final volume	Growth area, cm²/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 **Cell Quality Control Assay** and proceed by clicking the **Setup Assay** button.
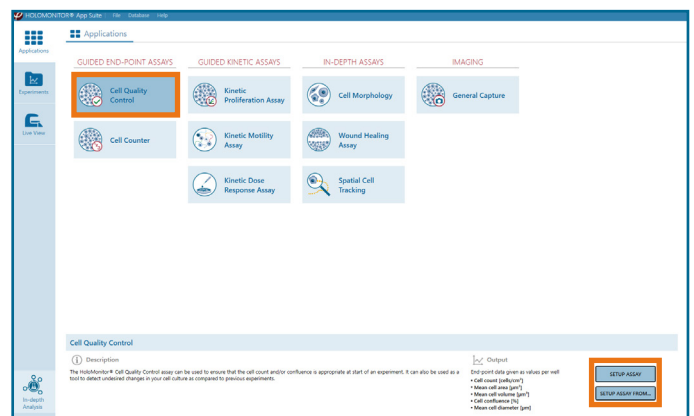
AppSuite main window with selected **Cell Quality Control Assay**

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**.
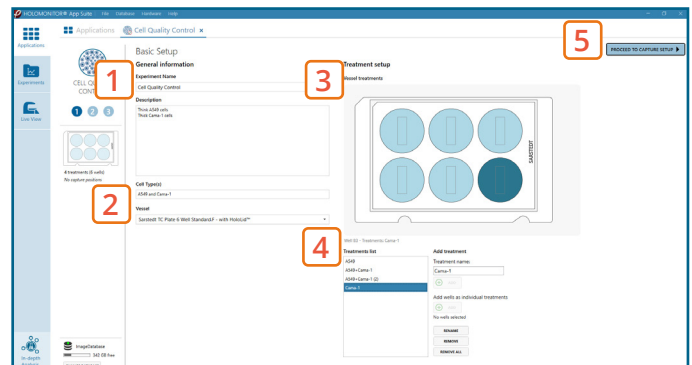
Basic Setup window

2 Capture setup: Select the experiment time settings and choose capture positions

1. The **number of positions** is set by default and can be adjusted.
2. Ensure that the **storage requirement** for the experiment does not exceed the computer capacity.
3. Click the **positions list** button to view the position list.
4. **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.
5. When satisfied with the experiment setup, click **Proceed to Capture**.
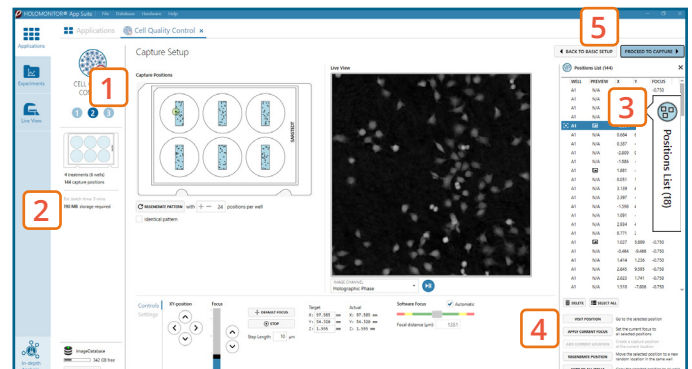
Capture Setup window

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 Operational 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.
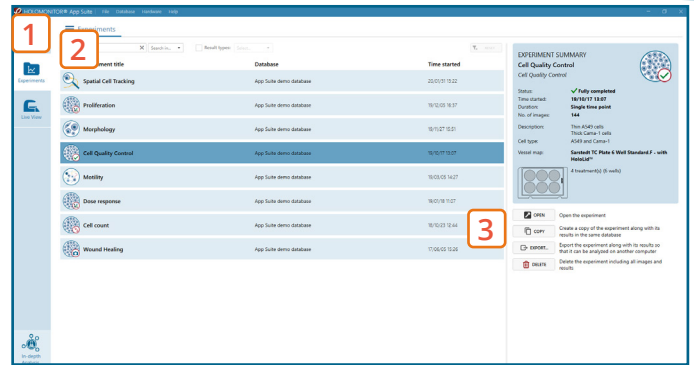4. When the Experiment capture finishes, click the **Show Result** button to get directly to the **Results page**.
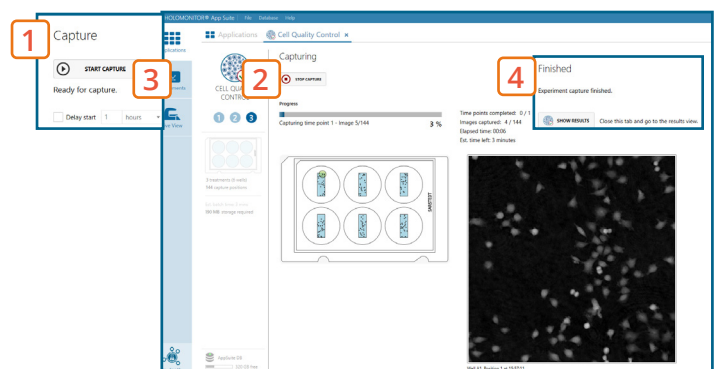
Capture window
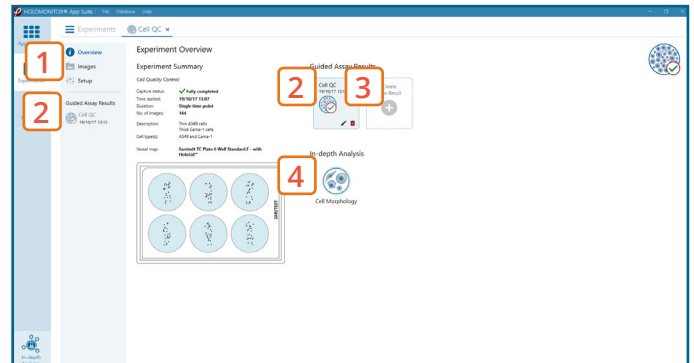
RESULTS & ANALYSIS

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.

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.
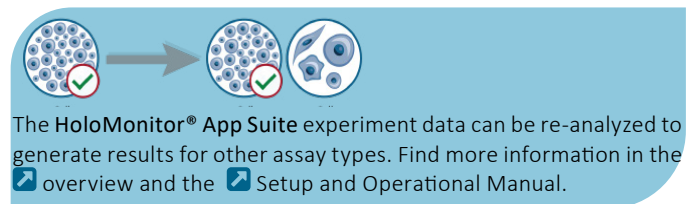4. Reach **In-depth Analysis** by clicking the respective icon.

Cell quality control 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 for each selected well/treatment are presented in a **table** showing cell count (#/cm^2), confluence (%), mean cell volume (μm^3), mean cell area (μm^2) and mean cell diameter (μm).
2. Enlarge the histograms to have a closer look at cell volume (μm^3) and area distribution (μm^2).
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.

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.
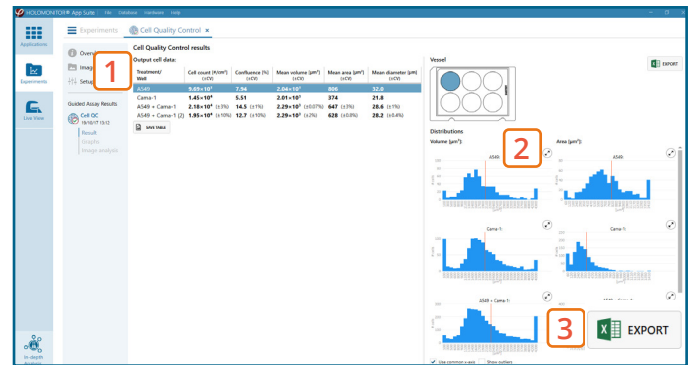

Experiments tab

Experiment overview tab

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 Operational Manual.

Cell Quality Control results tab

If **Coefficient of Variation (CV)** values are outside acceptable range user is advised to inspect image quality as described in ↗ Setup and Operational Manual, Image Analysis section.
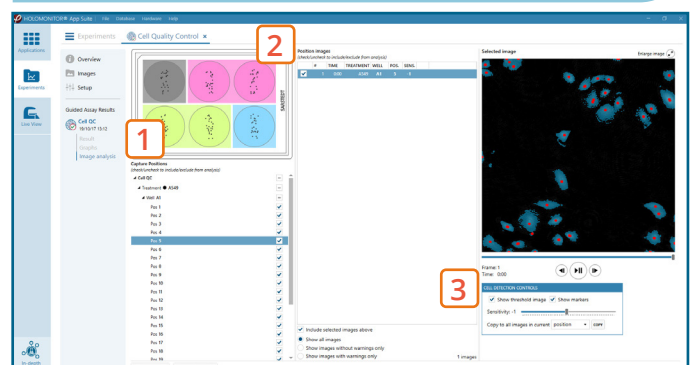

Image analysis tab