

HOLOMONITOR® APP SUITE PROTOCOL

CELL COUNTER

This protocol helps to set up a **Cell Counting Assay** using **HoloMonitor® M4** and the **HoloMonitor® App Suite** software. The **HoloMonitor® Cell Counter** is designed for automated cell counting of suspended cells. It can be used to prepare for seeding or for end-point cell counting of suspension cells. A **dilution calculator** provides the volume needed for homogeneous cell seeding.

REQUIREMENTS:

- HoloMonitor® M4
- HoloMonitor® M4 App Suite
- Countess cell counting chamber with suspended cells
- Vessel holder for microslides

OUTPUT:

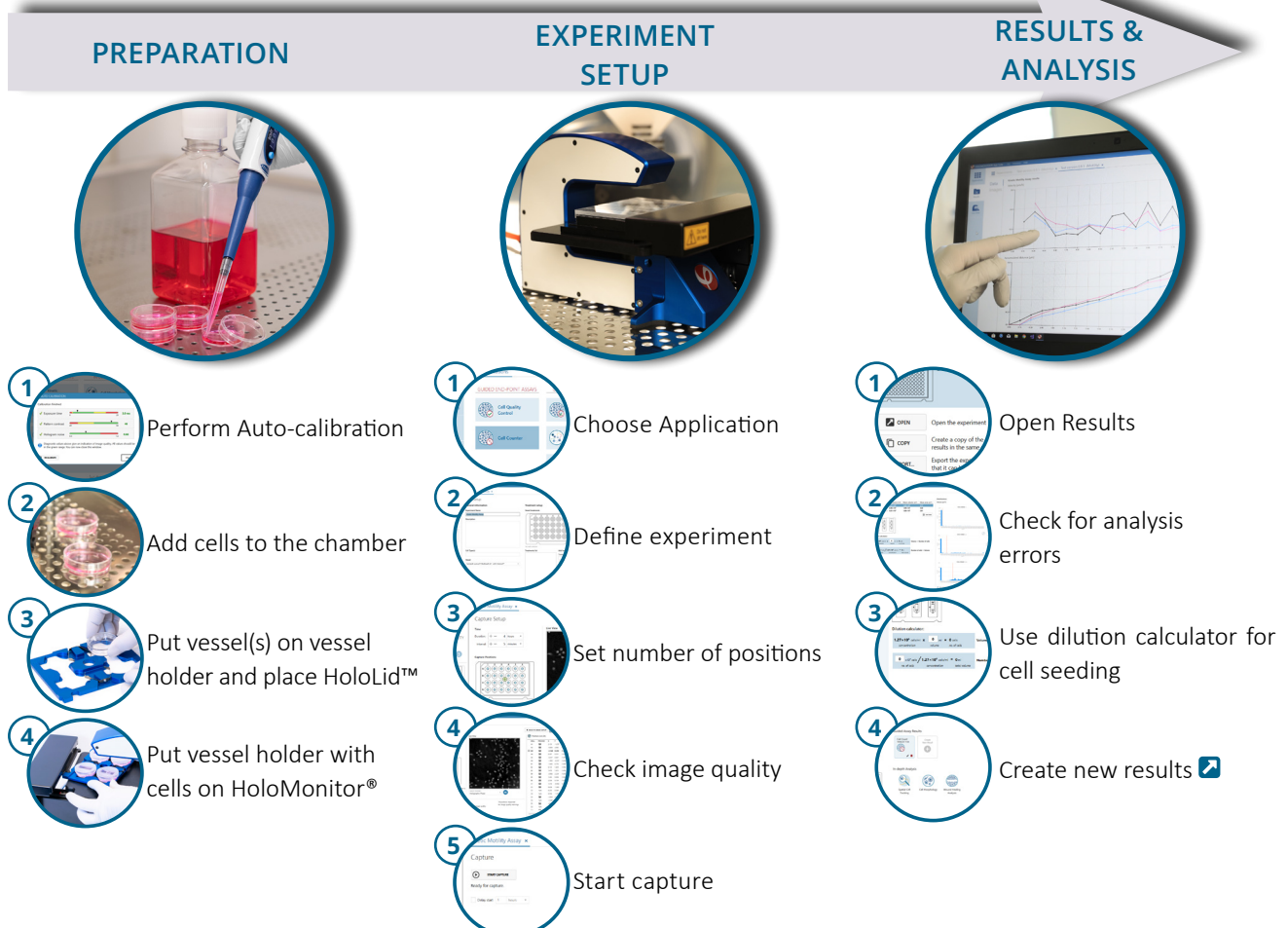
- Cell count (cells/mL)
- Mean cell volume (μm^3)
- Mean cell area (μm^2)

REANALYSIS:

- In-depth assays




WORK FLOW:



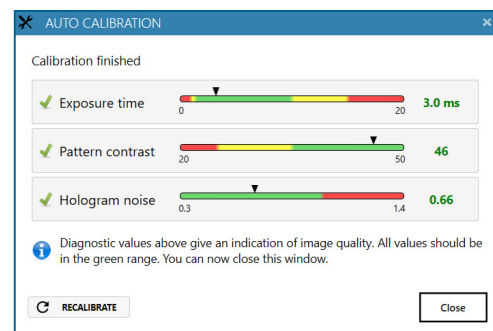
PREPARATIONS

Materials

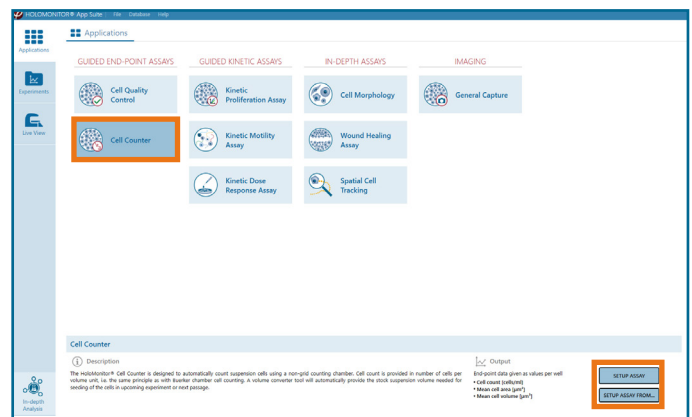
- ✓ HoloMonitor® M4
- ✓ HoloMonitor® App Suite software
- ✓ Cell culture vessel. Invitrogen Countess cell counting chamber slide (cat. # 10399053)
- ✓ Vessel holder for microslides
- ✓ Cells in suspension
- ✓  Setup and Operational Manual for HoloMonitor® M4

Steps

1. Start the software and wait for complete instrument initialization.
2. Run an auto-calibration. With successful calibration, the instrument is ready to use.
3. Add the cells with a concentration of $0.3 - 5 \times 10^6$ cells/mL in 10 μ L suspension to the counting chamber.
 - ▶ Please note that too few cells may cause inadequate results due to auto-focus failure.
4. Slide the counting chamber(s) into the Vessel holder.
5. Put the Vessel holder with the sample on the HoloMonitor® M4 stage.
6. Select the Cell Counter Assay and proceed by clicking the Setup Assay button.



Successful auto-calibration window

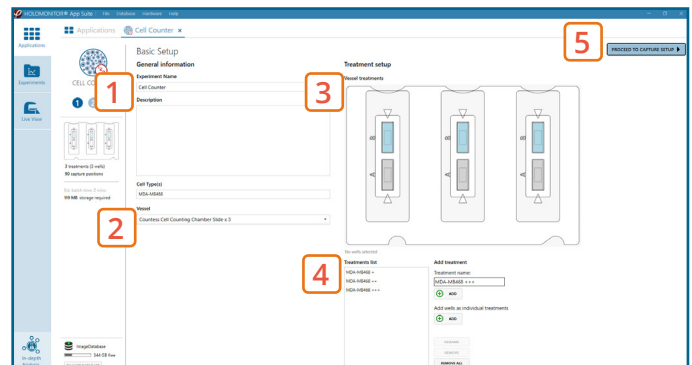


AppSuite main window with selected Cell Counter Assay

EXPERIMENT SETUP

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

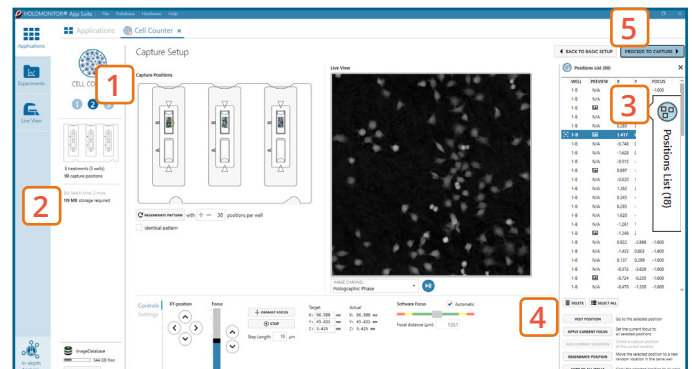
1. Enter the experiment **name**, optional experiment **description** and cell types.
2. The **vessel map** is pre-set to the **Countess Cell Counting Chamber Slide × 3**.
3. Map **treatments and conditions** on the vessel map. **Select wells** by marking them with the left mouse button while moving 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**.



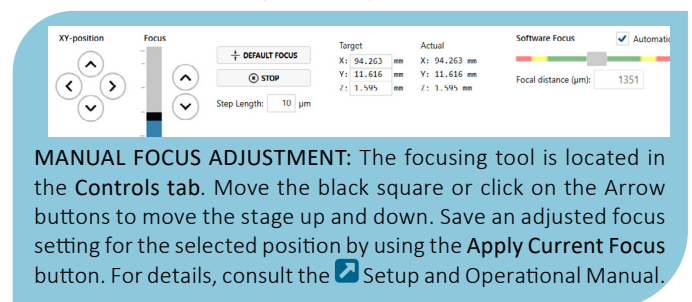
Basic Setup window

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**.

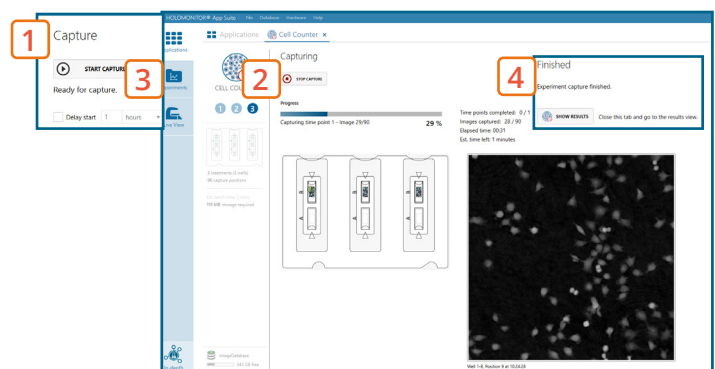


Capture Setup window



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.

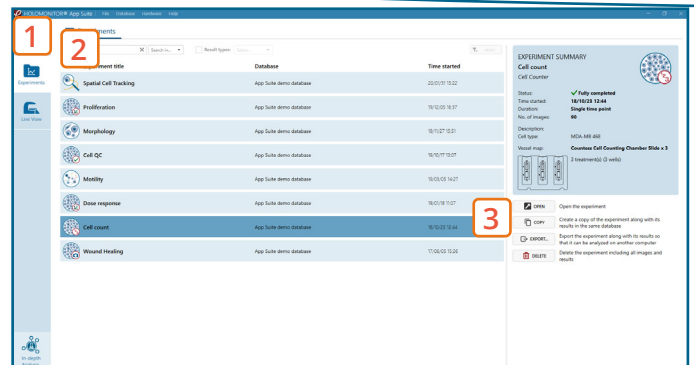


Capture window

RESULTS & ANALYSIS

Experiments tab

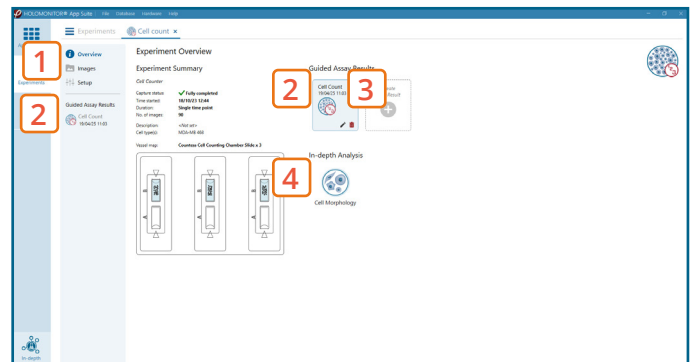
1. Click Experiments to see a list of the experiments.
2. Click on the experiment title to open an experiment summary.
3. Click Open to open the results page.



Experiments tab

Experiment overview tab

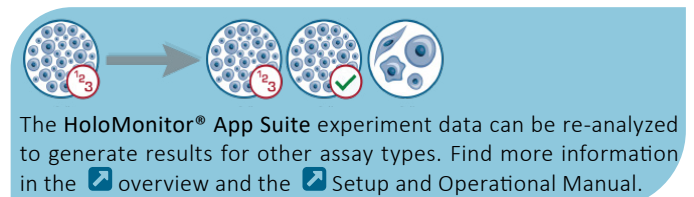
1. See the experiment summary, view all images and go to the experiment setup by choosing the respective tab.
2. Open the experiment data by clicking on the experiment result icon or its name in the Guided Assay Result list.
3. Create New Results from this experiment by clicking the button.
4. Reach In-depth Analysis by clicking the respective icon.



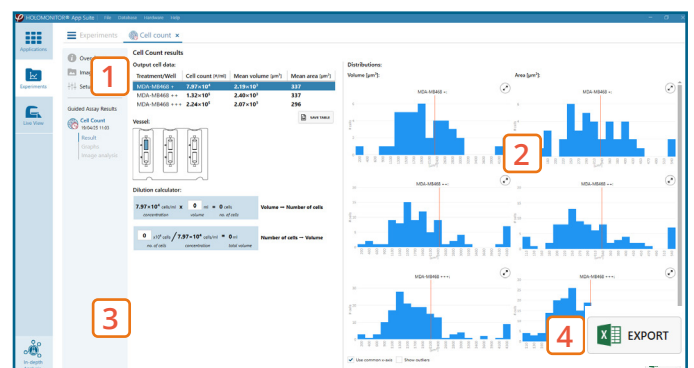
Experiment overview tab

Cell counter 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 (#/mL), mean cell volume (μm^3) and mean cell area (μm^2).
2. Enlarge the histograms to have a closer look at cell volume (μm^3) and area distribution (μm^2).
3. Use the dilution calculator to easily determine the cell number or volume needed for cell seeding.
4. 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.



Cell Counter results tab

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.

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.

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.

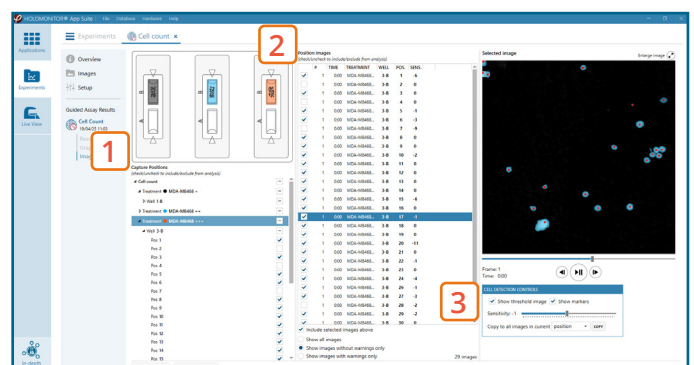


Image analysis tab