



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:

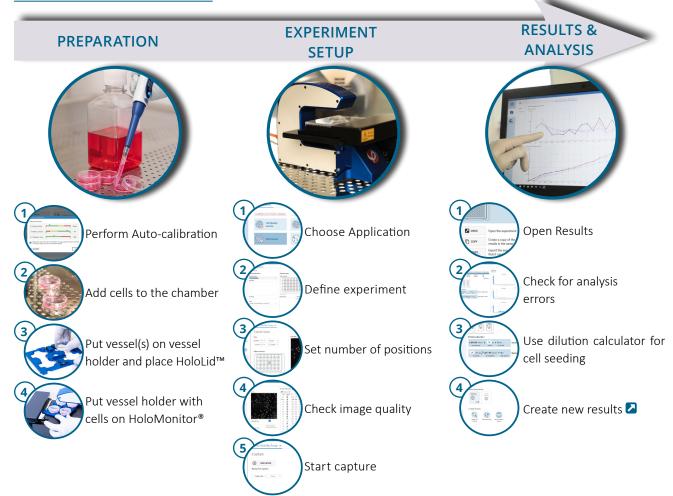
- Cell count (cells/mL)
- Mean cell volume (μm³)
- Mean cell area (μm²)



• In-depth assays



WORK FLOW:





PREPARATIONS

Materials

- ✓ HoloMonitor[®] M4
- ✓ HoloMonitor[®] App Suite software
- ✓ Cell culture vessel. Invitrogen Countess cell counting chamber slide (cat. # 10399053)

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.
- Add the cells with a concentration of
 0.3 5 x 10⁶ cells/mL in 10 μL 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.

- ✓ Vessel holder for microslides
- ✓ Cells in suspension
- ✓ Setup and Operational Manual for HoloMonitor ®M4

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Successful auto-calibration window

	OR® App Suite File Database Help				
cations	GUIDED END-POINT ASSAYS	GUIDED KINETIC ASSAYS	IN-DEPTH ASSAYS	IMAGING	
enents.	Cell Quality Control	Kinetic Proliferation Assay	Cell Morphology	General Capture	
ik. Sear	Cell Counter	Kinetic Motility Assay	Wound Healing Assay		
		Kinetic Dose Response Assay	Spatial Cell Tracking		
	Cell Counter				
	(j) Description			W Output	
ŝ	The HoloMonitor® Cell Counter is designed to a volume unit, i.e. the same principle as with laver seeding of the cells in upcoming experiment or ne	ker chamber cell counting. A volume converter to	-grid counting chamber. Cell count is provided i ool will automatically provide the stock suspensi	n volume needed for n volume needed for volume needed for Mean cell area (jum) ¹ Mean cell area (jum) ²	allo Ashi

AppSuite main window with selected Cell Counter Assay



EXPERIMENT SETUP

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

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- 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.
- 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.
- Name
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 Image: Supple state
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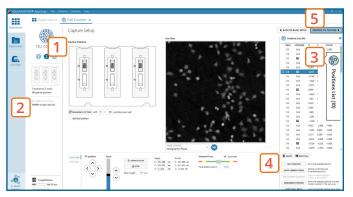
 Image: Supple state
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Basic Setup window

5. Proceed to Capture setup.

(2)Capture setup: Select the experiment time settings amd 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.
- 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 A appears. Adjust position location or focus if necessary.
- 5. When satisfied with the experiment setup, click **Proceed** to Capture.



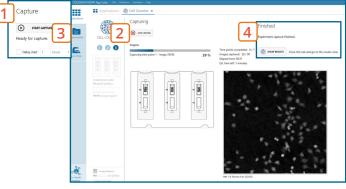




MANUAL FOCUS ADJUSTMENT: The focusing tool is located in the Controls tab. Move the black square or click on the Arrow buttons to move the stage up and down. Save an adjusted focus setting for the selected position by using the Apply Current Focus button. For details, consult the Setup and Operational 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.
- 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.

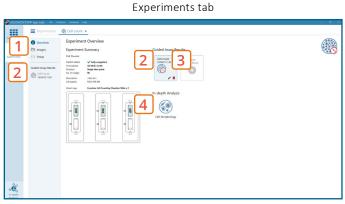
Experiment overview tab

- 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 counter data results tab
- \checkmark The assay results are generated during image capturing.
- ✓ The software automatically analyzes quantitative data generated from each image.
- $\checkmark~$ For a more detailed analysis all results can be exported.
- The results for each selected well/treatment are presented in a table showing cell count (#/mL), mean cell volume (μm³) and mean cell area (μm²).
- 2. Enlarge the histograms to have a closer look at cell volume (μ m³) and area distribution (μ m²).
- 3. Use the **dilution calculator** to easily determine the cell number or volume needed for cell seeding.
- 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.
- 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 (1) or image quality (1) warning.
- 3. It is possible to adjust the Sensitivity of image(s) and view the image threshold and markers.

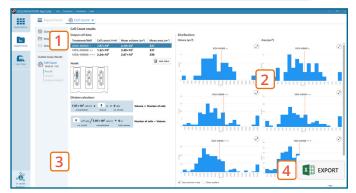
_	4		T _a and	EXPERIMENT SUMMARY
<	ment title	Database	Time started	Cell count Cell Counter
ments	Spatial Cell Tracking	App Suite demo database	20/01/31 13:22	C.
	Proliferation	App Suite demo database	16/12/05 18:37	Status ✓ Fully completed Time started: 18/18/23 12:44 Durston: Single time point No. of images: 90
	Merphology	App Suite demo database	19/19/27 15:51	Description Cell type: MDA. MB. 458
	cui qc	App Suite demo database	15/10/17 12:07	Vectel map: Countees Cell Counting Chamber Slide x
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	🛞 Cel court	App Suite demo database	10221244 3	Cover Create a copy of the experiment along with its results in the same database Diport the experiment along with its results so
	Wound Healing	App Suite demo database	17/06/05 15:25	Deport the experiment along with its results so that it can be analyzed on another computer Delete the experiment including all images and results



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 Counter 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.

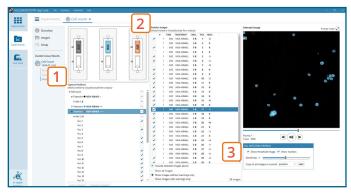


Image analysis tab