IMAGE QUALITY GUIDE

This image guide intends to assist HoloMonitor® M4 user when evaluating image quality during experiment setup and result analysis.

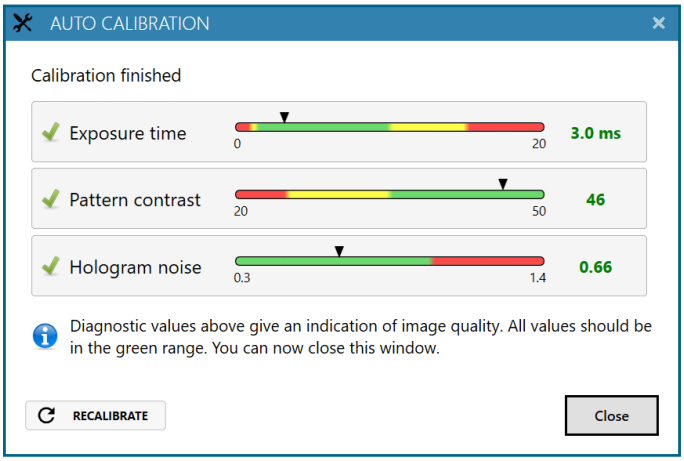
Please contact support@phiab.com if you need further assistance assessing image quality.

Experiment Setup

Before you start

1. Check the **status of your cells**: image a **maximum of 80% confluency** in a field of view to allow the software algorithm to identify individual cells from the background. HoloMonitor® M4 is designed to visualize and quantify **adherent monolayer cell cultures**, therefore your cells should not grow in multiple layers or in suspension cell culture.

2. Always perform **Auto-calibration** before every experiment. The calibration minimizes background noise.



For a susscesful auto-calibration, all values (indicated by black arrow heads) should be in the green range.

Reasons for failed auto calibration:

- If **all values are** in the **red region** - laser does not produce enough light. Reasons: dirty optics, laser being not properly connected or dirty, laser requires periodical recalibration. For resolving issues, consult the Setup and Operation Manual.
- **If Hologram noise is in the red range** - HoloMonitor® M4 might have dirt on the optics or dirt inside the instrument. Clean the objective, if the problem presists, contact technical support (by writting email to support@phiab.com) for further assistance.

During Capture Setup

Inspect the images when setting up the capture for the experiment. Run a full or quick **validation** to **ensure good image quality**. If the image quality is poor, a warning sign appears.

Having cells grow too sparsly may introduce image analysis warnings. Make sure there are **at least a few cells** in the field of view. **Adjust** the capture position location if necessary.

Reasons for poor image quality could be: incorrect focus, condensation, dirt and particles, air bubbles, smears and scratches, or vessel's optical properties.

Below are the examples of poor quality images prompted due to various reasons:

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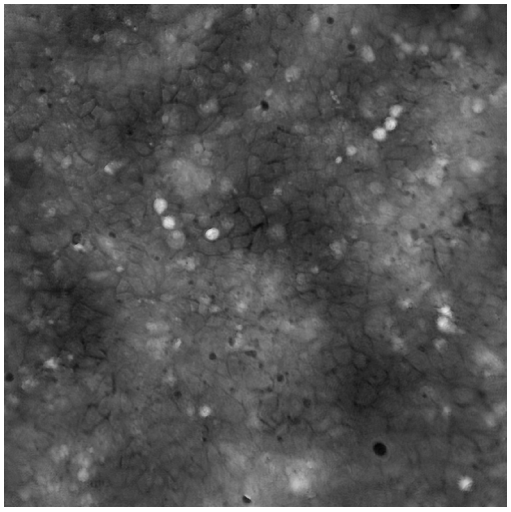
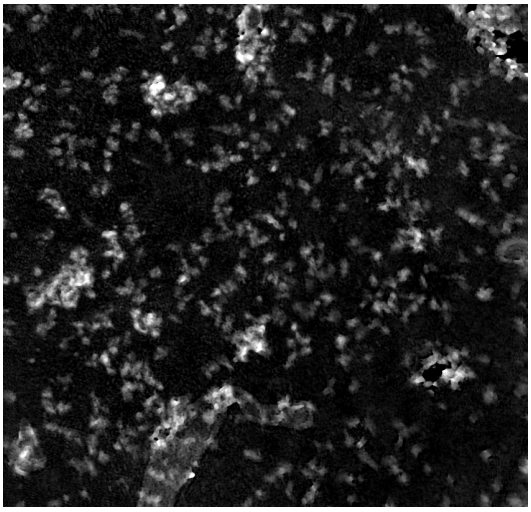
Cell are growing in multilayers or in suspension

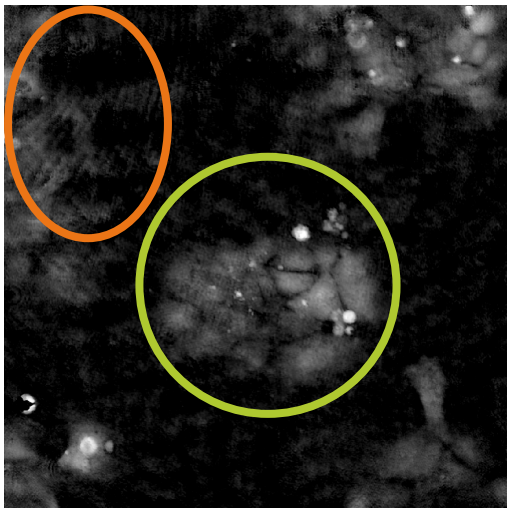


Cell growing in multiple layers cannot be identified or quantified.



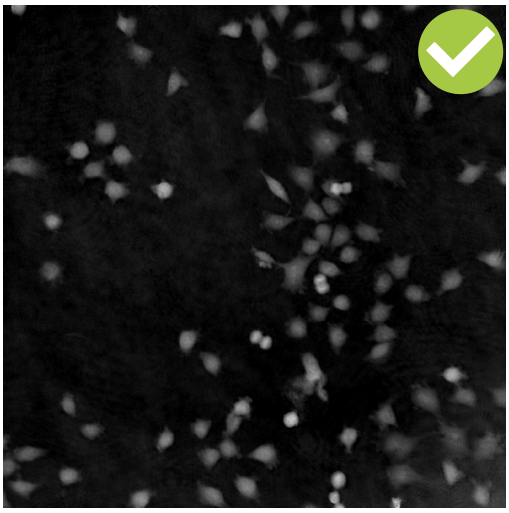
Cells growing in suspension cannot be focused, tracked and quantified.

High background noise

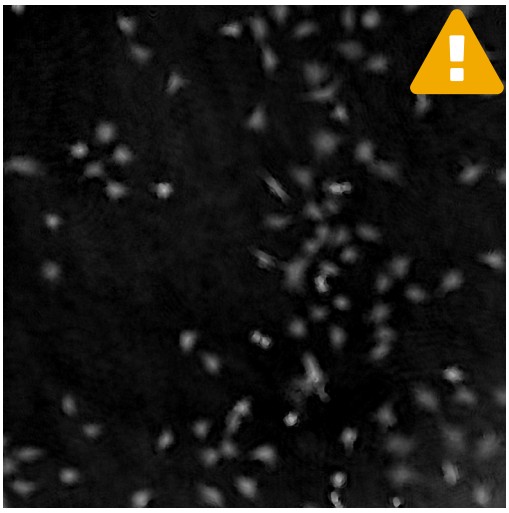


Thin cells (green circle) and strong background noise (orange circle). A minimal background noise is especially important when working with very thin cells to distinguish cells from a strong background noise.

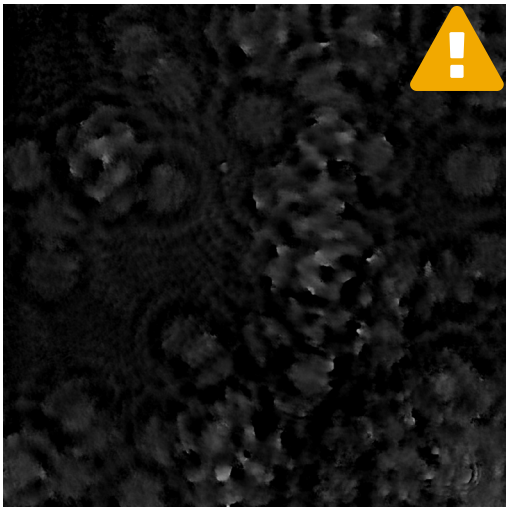
Incorrect focus



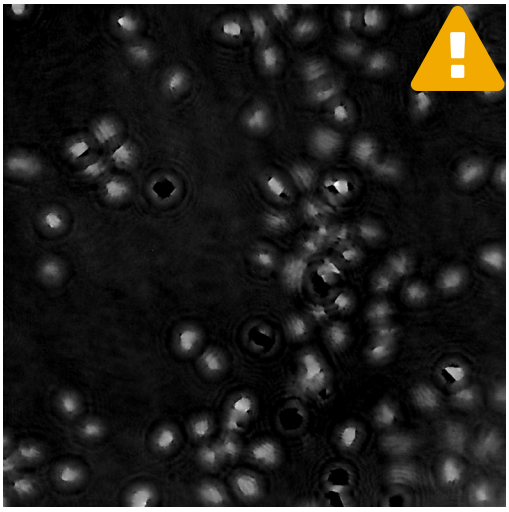
Correct focus



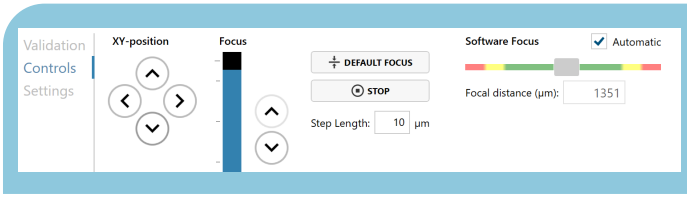
Incorrect focus



Incorrect focus

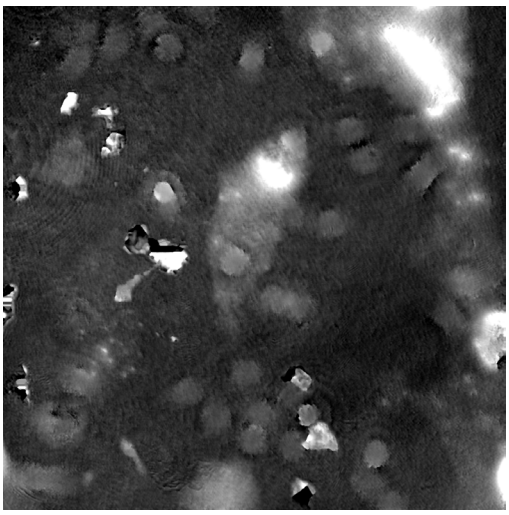


Incorrect focus

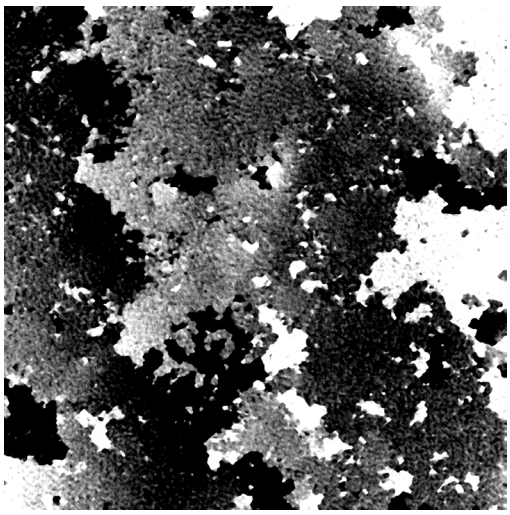


MANUAL FOCUS ADJUSTMENT: The focusing tool is located in the **Controls tab**. Move the black square or click on the Arrow buttons to move the stage up and down. Save an adjusted focus setting to the selected position by using the **Apply Current Focus** button. For details, consult the Setup and Operation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

Condensation



Some condensation obstructing field of view

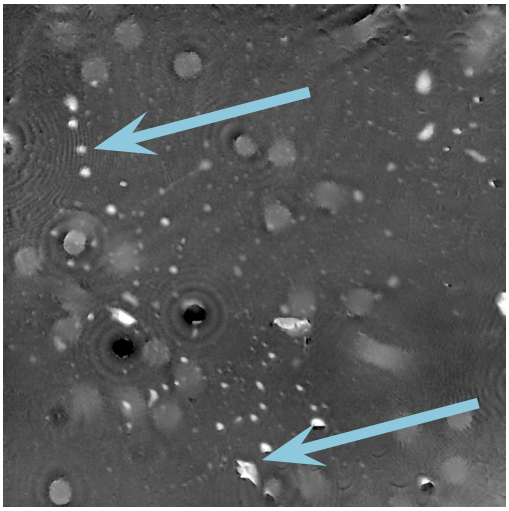


Field of view fully obstructed by condensation

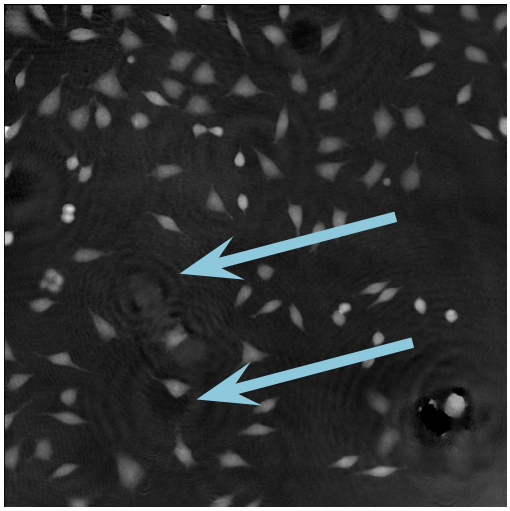
Tips to reduce condensation:

- **Use pre-warmed cell culture media**, as the cold one introduces a high probablity of condensation forming outside of the vessel. The condensation disappears once the media in a well is warm (1-2 hours after addition).
- **Use HoloLids™** to eliminate image disturbances caused by condensation and surface vibrations inside the cell culture vessel. Details and user instructions are available in HoloLids™ protocol.

Dirt and Particles



Particles

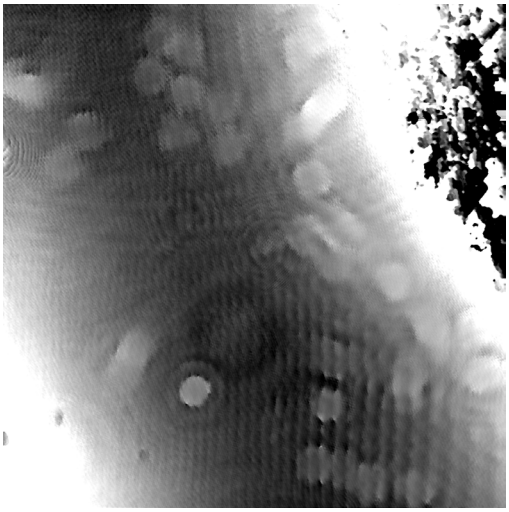


Dirt/dust creates “swirly patterns“ in the image

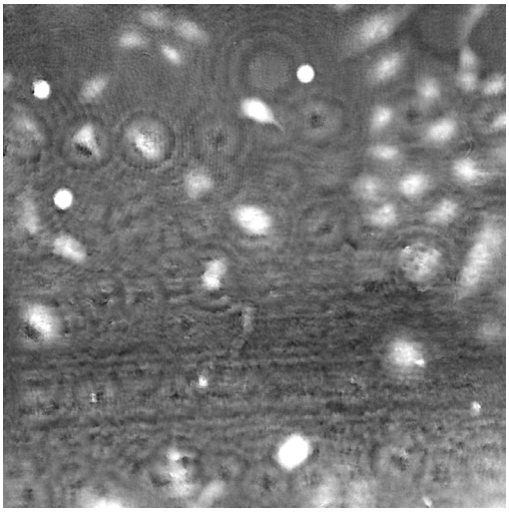
Tips to avoid dirt and particles:

- **Never use paper or fabric** to remove liquid drops or condensation. Fibers will stick onto the cell culture vessel.
- Always **work in a clean environment** - use LAF-bench to prevent dirt particles getting into the cell culturing media.
- **Clean HoloMonitor® M4 outer surfaces** accoding to Setup and Operation Manual.
- It is recommended to **service HoloMonitor M4 yearly**. For more information contact technical support (by writting email to support@phiab.com) or your local distributor.

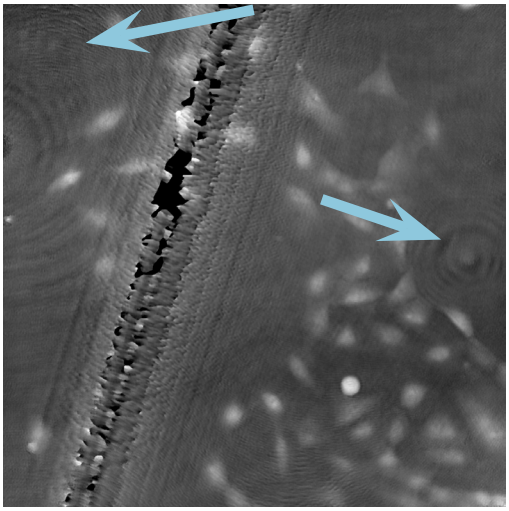
Airbubbles, smears and scratches



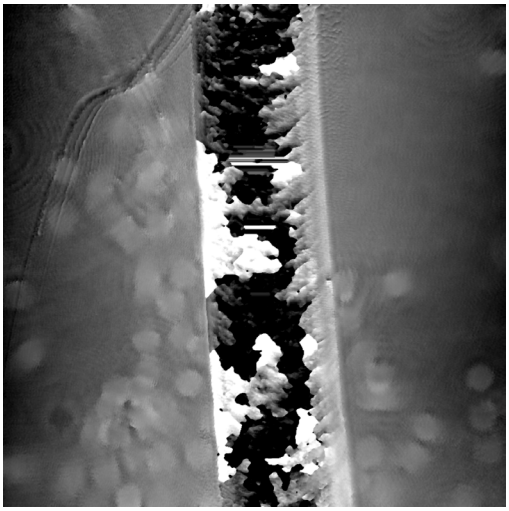
Airbubble at the top right corner



Smear



Thin scratch and floating cells (blue arrows)

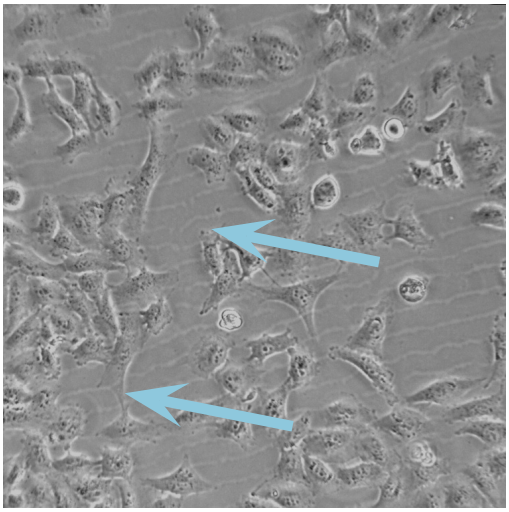


Thick scratch

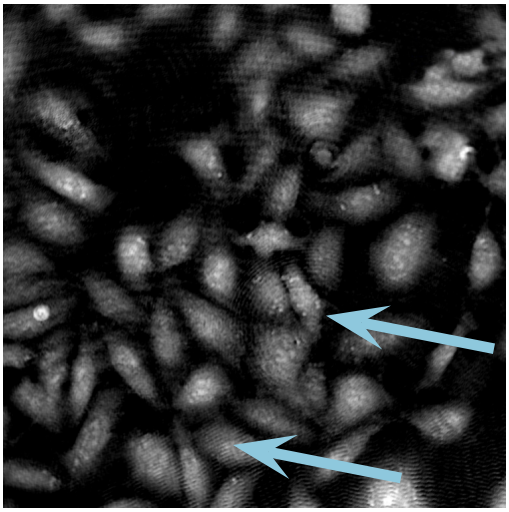
Tips to prevent airbubbles, smears and scratches:

- **To remove airbubbles use clean air**. This can be created by using an ethanol dispensing bottle with inner tube removed, and which contains on a small volume (100-150 mL) of 70% ethanol. Press the bottle carefully while directing the air stream towards the bubble. Do not touch cell media.
- **To prevent smears** - always wear laboratory gloves.
- **To prevent smears and scratches** - only hold cell culture vessel at its sides and do not touch the vessel bottom or lids.

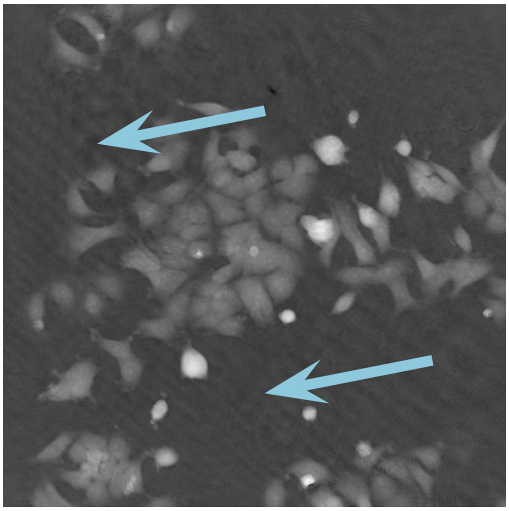
Low optical quality plastics of cell culture vessels



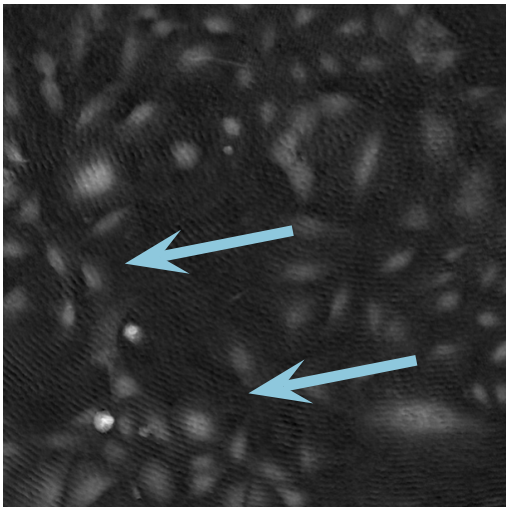
Plastic interference lines visible in bright field



Plastic interference lines visible in holographic image



Plastic interference lines visible in holographic image



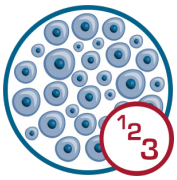
Plastic interference lines visible in holographic image

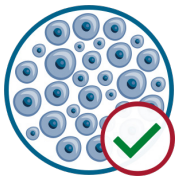
Tip to avoid low quality plastic:

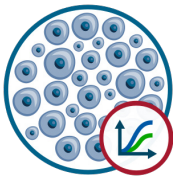
- **Consult HoloMonitor® M4 recommended cell culture vessel list** to learn more about which vessels have been tested and are compatible with HoloMonitor® M4.

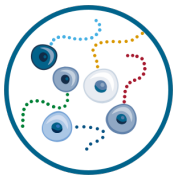
Result Analysis

Guided Assays


Cell Counter


Cell Quality Control


Kinetic Proliferation Assay


Kinetic Motility Assay


Kinetic Dose Response Assay

When the capture finishes, click the Show Result button to get directly to the Experiment Results page.

The assay results are generated during image capturing. The software **automatically analyzes** quantitative data generated from each image.

Image analysis tab

✓ An image analysis algorithm filters out images of poor quality and excludes them from results data.

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.

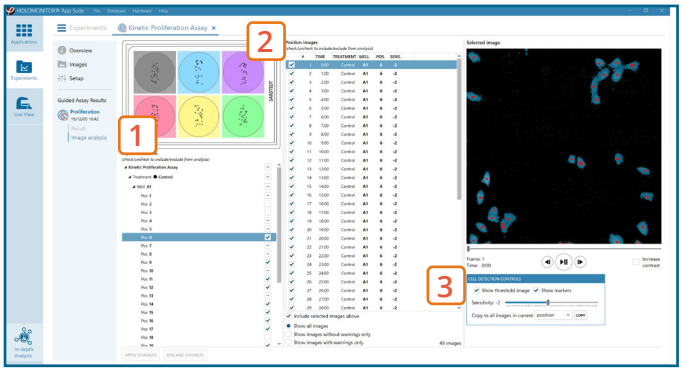
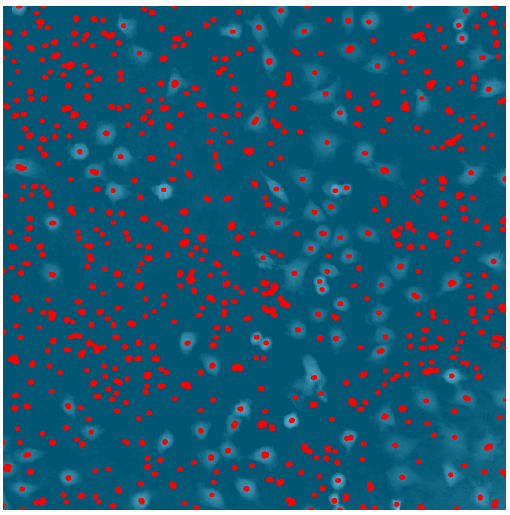


Image analysis tab

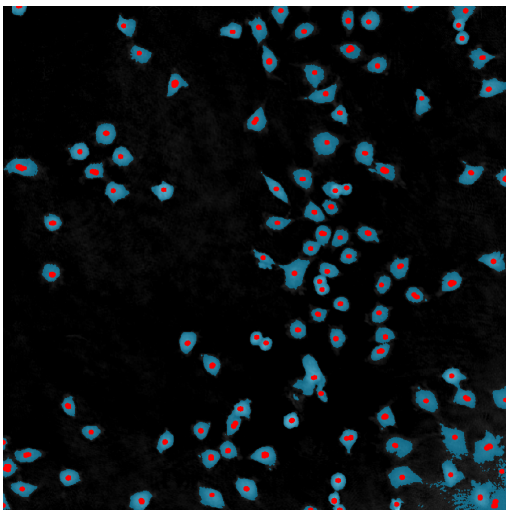
- It is **users responsibility to verify** that the software algorithm was correctly applied during image analysis and that results are accurate.
- User can choose to **adjust sensitivity of the image threshold**, and to **include** or **exclude** images from results analysis.
- For more information, see **Image Analysis Section** in the Setup and Operation Manual for HoloMonitor® M4 .

Below are some examples on how to improve image analysis by adjusting the sensitivity of the image and therefore the threshold of cell detection. Additionally cases, when the frames should be excluded from the analysis by unchecking the check box, are shown.

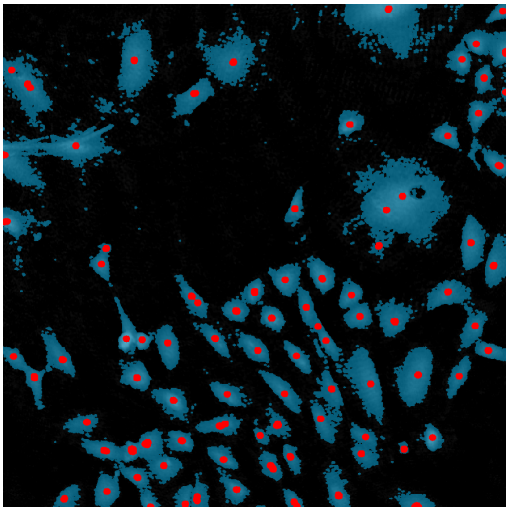
Adjust cell detection sensitivity



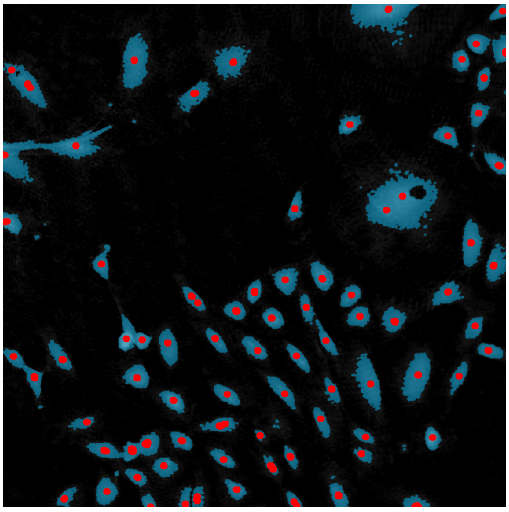
Sensitivity mask covers all cells, although the cells are not confluent.



After sensitivity adjustmensts, the mask covers individual cells only.

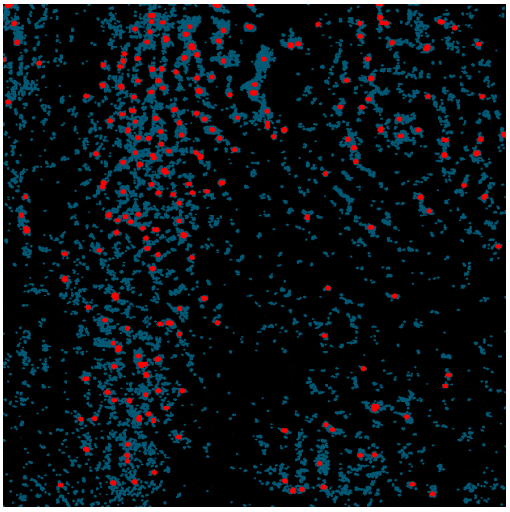


Sensitivity mask covers some of the background

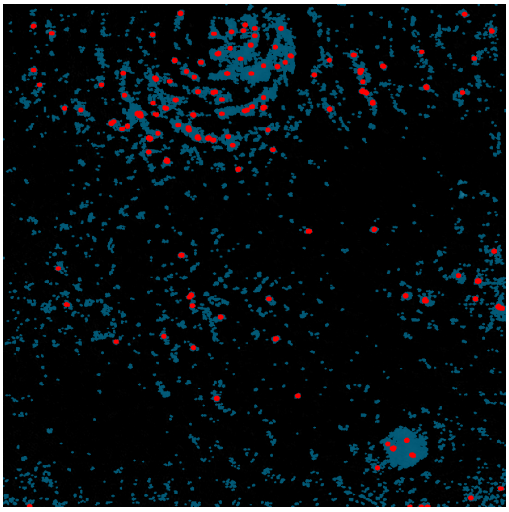


After sensitivity adjustmensts, the mask covers individual cells only - their outlines are clear and background noise is minimized.

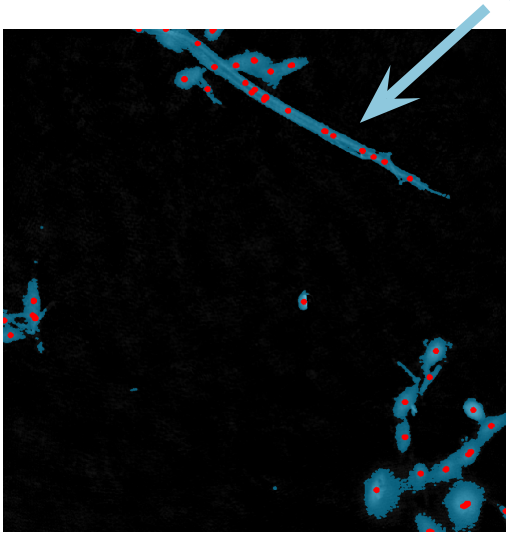
Examples of frames that should be excluded from Analysis



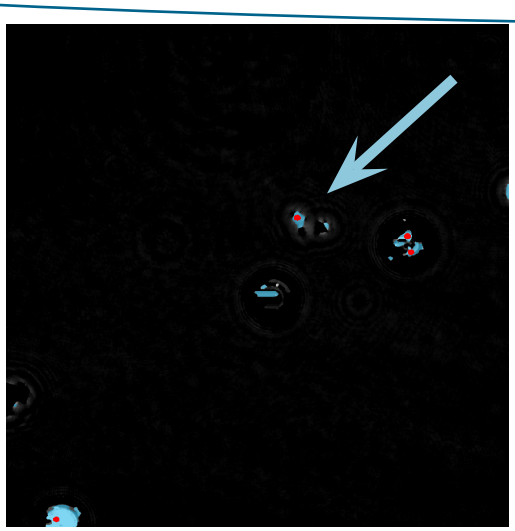
No cells or too few cells in the field of view



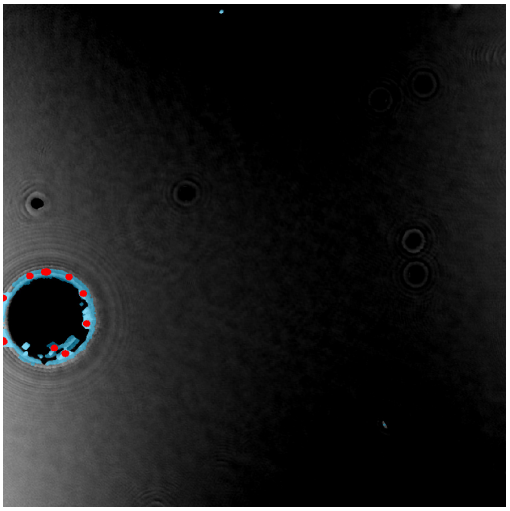
No cells or too few cells in the field of view



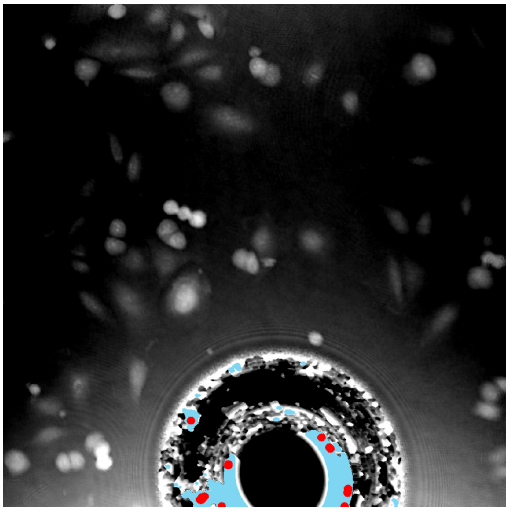
Fillament/dust disturbing the frame



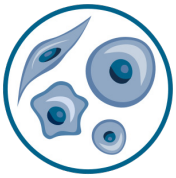
Cells are out of focus



Small airbubble and no cells in the field of view



Airbubble disturbs the mask, thus cells are not covered



Cell Morphology

Single Cell Tracking

When the Experiment capture finishes, click the Show Result button to get directly to the Experiment Overview page.

Generate in-depth analysis data from the captured images by selecting one of the applications icons.

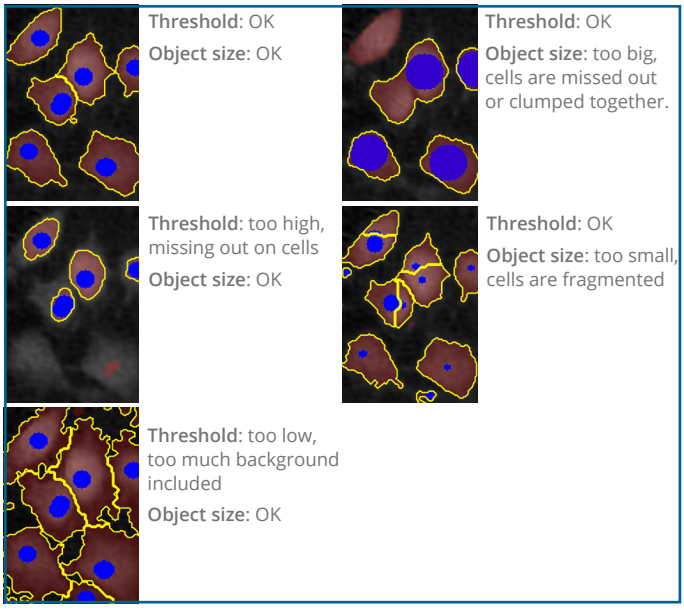
In-depth analysis - Identify cells tab

✓ First step is to identify the cells.

✓ Inspect the image quality and include/exclude frames from the analysis.

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 cell markers or grow/ shrink a cell outline, delete a cell area or draw a cell area yourself. These changes will **only** be saved to the **currently selected image**.
4. View the full **Cell Morphology** and **Single Cell Tracking** protocols for further in-depth analysis steps.

Identify cells tab



Threshold: OK
Object size: OK

Threshold: OK
Object size: too big, cells are missed out or clumped together.

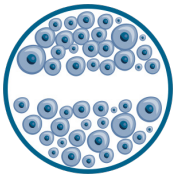
Threshold: too high, missing out on cells
Object size: OK

Threshold: OK
Object size: too small, cells are fragmented

Threshold: too low, too much background included
Object size: OK

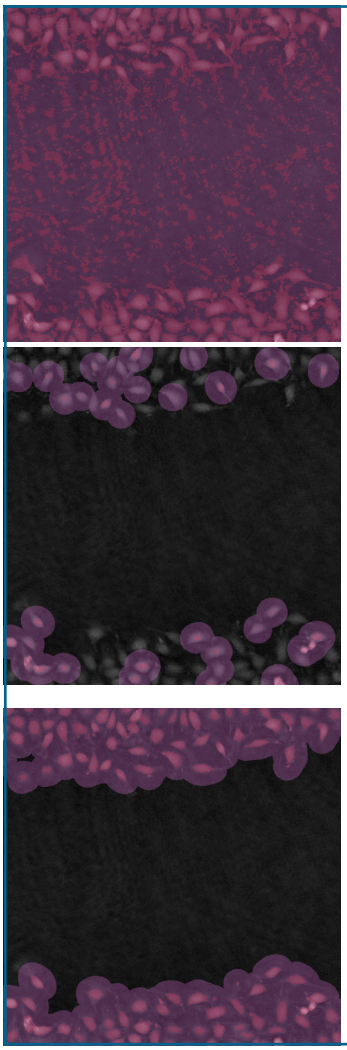
- It is **users responsibility to verify** that the software algorithm was correctly applied during image analysis and that results are accurate.
- Check cell segmentation for images throughout the experiment to make sure the applied threshold fits.
- The more thorough user is in this step, the better analysis results are in the following steps.
- It is important for in-depth analysis that identifying the cells is done correctly.
- For more information, see **Identify cells** in the Setup and Operation Manual for HoloMonitor® M4 .

In-depth analysis - Wound healing tab



Wound Healing Assay

1. Check the image quality before adding frames to the Wound healing analysis.
2. **Adjust** the image threshold and object size and click **Apply to all frames**.
3. View the full **Wound Healing** protocol for further in-depth analysis steps.



Threshold: too low, too much background included

Threshold: too high, missing out on cells

Threshold: OK

- It is **users responsibility to verify** that the software algorithm was correctly applied during image analysis and that results are accurate.
- Check the mask coverage for all the images throughout the experiment to make sure the applied threshold fits correctly.