Phase Holographic Imaging

HoloMonitor® M4 PROTOCOL

PROLIFERATION GROWTH CURVES

June 2017, rev. 2

MATERIAL

- ***HoloMonitor® M4***, placed inside a cell incubator.
- ***Culture vessels*** by choice: Sarstedt 6-well (growth area 8.87 cm^2/well), Sarstedt 96-well plate with Lumox bottom (growth area: 34 mm^2/well), Sarstedt 24-well plate with Lumox bottom (growth area: 190 mm^2/well) or Sarstedt 35 mm cell culture dish (growth area: 8 cm^2).
- ***Vessel holder*** for the selected vessel.
- ***Cells*** suspended to their adequate concentration to reach a confluence of 3-10 % when seeded, depending on cell line. We have used L929 cells[1], approximately 6000-11 000 cell/cm^2 to reach a confluence of 3-10 % when newly seeded (round with small areas).
- ***HoloLids*** (www.phiab.se/products/hololids).
- ***Setup and Operational manual*** for using HoloMonitor (www.phiab.se/support).

[1] We have used L929, A375, and Jimt-1 cells. Other cell lines may work equally well but the protocol must be optimized for them by the user.

PREPARATION

Day 0

1 Seed the cells to a confluence of 5-10% in the preferred vessel. The final working volumes, essential for using the HoloLids, are: 3 ml/well for 6-well plates and 35 mm cell culture dish, 170 µl/well for 96-well plates, and 1.8 ml/well for 24-well plates. Remember to take into account that the volume of the treatment adds to the final working volume.

2 Put the vessel into the cell incubator and let the cells attach for 24h.

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START-UP THE IMAGING

Day 1

1 Start up the ***HoloMonitor*** and proceed with the calibration. The values achieved should lie within the green area of the calibration results bar.

2 Add the treatment if stated in the experimental setup and change lids to ***HoloLid*** by following the lid protocol.

3 For imaging with a motorized stage:

3.1 Place the plate, slide or dish in to the vessel holder and thereafter the holder on to the ***HoloMonitor stage*** by clicking it on sideways on to the “half-moons” on the stage, right side first.

3.2 Go to the ***Live capture*** tab in the ***Hstudio software*** and select the adequate vessel template.

3.3 Create a ***Project*** for image storage.

3.4 Focus the images at a position close to the center of the plate/vessel.

3.5 Click ***Time-lapse*** and type the total time and interval of the time-lapse imaging. 6-12 hours between captures is recommended.

3.6 Click ***Capture pattern*** and select the wells to be captured.

3.7 Select at least 5 capturing positions to capture for each sample. The more heterogenous the cell distribution, the more captures are needed.

3.8 Go to capturing positions at the four corners of the vessel and ensure they are well focused (left-click the red squares in the stage position window).

3.9 Click ***Advanced setup*** and check ***Multiple destination groups***. Then check ***One group per well*** (default value). Click ***Save and close***.

3.10 ***Click Capture***. Go to the ***View image*** tab and review the images for quality check.

3.11 Wait for the multiple time-lapse capturing to finish.

4 For imaging with a fixed stage:

4.1 Create one ***Group*** per sample, i.e. “Control1 Day 1” and “Treated Day 1”.

4.2 Focus the image using the correct distance plate.

4.3 Capture at least 5 images per sample, the more heterogeneous the cell distribution, the more captures are needed.

4.4 Repeat capturing (step 4.1-4.3) for as many time points as the experiment requires.

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ANALYSIS

1 Go to ***Identify cells***. Check the segmentation and, if needed, adjust the ***Threshold*** and ***Min object size*** settings to fit the cells for all ***Groups***. The settings can be applied for all images within each ***Group*** but need to be validated and possibly adjusted for all ***Frames***. Discard bad frames.

2 Go to ***Identify cells***. Adjust the ***Threshold and minimum object size*** for all frames to fit most of the cells for each ***Group***. Use the same settings for each sample. Discard bad ***Frames***.

3 Go to ***Cell count***. Add the images corresponding to one time-point and one treatment, e.g. control at 0 hours (see image below).

4 Adjust the ***Vessel growth area*** (below, red arrow) to correspond to the vessels used.

5 Note the ***Number of cells in vessel***, the ***Confluence*** and ***Confidence interval*** (the number to the right of the cell number). If the number is above 10%, more frames ought to be added to the cell count to achieve sound statistics.

6 A ***Report*** (PDF) can be created by pressing the ***Save report*** button.

7 Repeat the cell count for all treatments and time-points.

8 Create graphs using the values from the cell count reports using e.g. ***Excel***.

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