

# HoloMonitor<sup>®</sup> M4 PROTOCOL

## PROLIFERATION GROWTH CURVES

April 2017, Rev. 1

### MATERIAL

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- **HoloMonitor<sup>®</sup> M4**, placed inside a cell incubator.
- **Culture vessels** by choice: Sarstedt 6-well (growth area 8.87 cm<sup>2</sup>/well), Sarstedt 96-well plate with Lumox bottom (growth area: 34 mm<sup>2</sup>/well), Sarstedt 24-well plate with Lumox bottom (growth area: 190 mm<sup>2</sup>/well) or Sarstedt 35 mm cell culture dish (growth area: 8 cm<sup>2</sup>).
- **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<sup>1</sup>, approximately 6000-11 000 cell/cm<sup>2</sup> to reach a confluence of 3-10 % when newly seeded (round with small areas).
- **HoloLids and protocols for their use** ([www.phiab.se/products/hololids](http://www.phiab.se/products/hololids)).
- **Setup and Operational manual** for using HoloMonitor, if the user is unfamiliar with the imaging procedures.
- **Software manual**, if the user is unfamiliar with the Hstudio software.

<sup>1</sup>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

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#### 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.

## START-UP THE IMAGING

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### 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.

## 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.

**Cell Count Report**

Number of cells in vessel: **1.17e+006 ±14%**  
 Number of cells per ml: **2.34e+005**  
 Confluency: **29% ±4**

Report date: 27/01/2017 15:32  
 Capture at: 13/10/2016 10:44:01 - 10:46:54  
 Vessel growth area: 25 cm<sup>2</sup>  
 Vessel media volume: 5 ml  
 Total number of images: 15  
 Total imaged area: 4.816 mm<sup>2</sup>  
 Total number of cells in images: 2471  
 Number of cells on image edges: 438

Area (µm<sup>2</sup>) distribution

Volume (µm<sup>3</sup>) distribution

Vessel growth area: 25 cm<sup>2</sup>  
 Vessel media volume: 5 ml  
 Cell count per ml: [ ]  
 Area-histogram: [ ] Auto [ ] 60.5 [ ] 2704 µm<sup>2</sup> No. of bins: 45  
 Volume-histogram: [ ] Auto [ ] 125.7 [ ] 16027.1 µm<sup>3</sup> No. of bins: 45  
 Save report... [ ] Include histograms [ ] Include source frames list

Number	Group	Project	Comment
1	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (94.078; 23.586; 5.767) in A1
2	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (94.480; 13.098; 5.767) in A1
3	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (99.061; 13.382; 5.767) in A1
4	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (89.730; 12.833; 5.767) in A1
5	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (90.002; 18.033; 5.767) in A1
6	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (102.097; 11.902; 5.767) in A1
7	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (95.276; 20.009; 5.767) in A1
8	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (89.539; 19.174; 5.767) in A1
9	Capture#1 October 13 (Well A1)	Sebastian Test 1 JIMT-1 4E	Take #1: (91.753; 20.857; 5.767) in A1

- 5 Note the **Number of cells in vessel**, the **Confluency** 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**.