

# HoloMonitor® M4

#### PROLIFERATION GROWTH CURVES PROTOCOL

#### MATERIAL

- HoloMonitor<sup>®</sup> M4, placed inside a cell incubator.
- Hstudio software, version 2.7.1 or later.
- **Culture vessel** by choice:
  - Sarstedt TC Dish 35, Standard (cat. # 83.3900)
  - Sarstedt TC Plate 6 Well, Standard, F (cat. # 83.3920.005)
  - Sarstedt lumox<sup>®</sup> multiwell, 24 Well (cat. # 94.6000.014)
  - Sarstedt lumox<sup>®</sup> multiwell, 96 Well (cat. # 83.3924.005)
- PHI **HoloLid** for selected vessel:

Vessel	HoloLid
Sarstedt TC Dish 35, Standard	71110
Sarstedt TC Plate 6 Well, Standard, F	71120
Sarstedt lumox <sup>®</sup> multiwell, 24 Well	71130
Sarstedt lumox <sup>®</sup> multiwell, 96 Well	71140

HoloLid product information and protocol is available here.

- PHI **Vessel holder** for the selected vessel For information regarding vessel holders contact PHI at <u>support@phiab.se</u>.
- **Cells** suspended to reach a confluence of 2-5 % when seeded (approx. 6 000-11 000 cell/cm<sup>2</sup> for L929, A375, and Jimt-1 cells). Other cell types may require a different seeding concentration.
- <u>Setup and Operation Manual</u> for using HoloMonitor M4, if the user is unfamiliar with the imaging procedures.

We recommend that each experiment is performed at least three times to acquire sound statistics.





### PREPARATION

1. Seed the cells to a confluence of 2-5 % in preferred vessel. The final working volumes, essential for using *HoloLids* are:

Volu	ume	Vessel
3.0	ml	Sarstedt TC Dish 35, Standard
3.0	ml/well	Sarstedt TC Plate 6 Well, Standard, F
1.8	ml/well	Sarstedt lumox <sup>®</sup> multiwell, 24 Well
170	µl/well	Sarstedt lumox <sup>®</sup> multiwell, 96 Well

- 2. Put the vessel into the cell incubator and let the cells attach for 2-24h.
- 3. Sterilize the *HoloLids* according to the *HoloLid protocol*.
- 4. Add the treatment, if stated in the experimental setup.
- 5. Put on the standard lid.

## IMAGING

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

#### For imaging with a motorized stage

- 1. Wipe off the *Vessel holder* with alcohol and put it in to the LAF-bench, the grips facing down.
- 2. Place the cell samples on to the *Vessel holder*:



3. Replace the standard lids with the appropriate *HoloLid*, following the HoloLid protocol.



4. Thereafter place the *Vessel holder* with the samples on the HoloMonitor stage:



5. Go to the *Live capture tab* in the Hstudio software and select the appropriate vessel template:

Vessel	Template
Sarstedt TC Dish 35, Standard	Petri dishes 40 mm
Sarstedt TC Plate 6 Well, Standard, F	Sarstedt 6 well plate with PHI lid
Sarstedt lumox® multiwell, 24 Well	Lumox 24 well plate
Sarstedt lumox® multiwell, 96 Well	Lumox 96 well plate with PHI lid

- 6. Create a *Project* for image storage.
- 7. Focus the images at a position close to the center of the plate/vessel.
- 8. Check *Timelapse* and type the total time and interval of the time-lapse imaging. 6-12 hours between captures is recommended.
- 9. Check *Capture pattern* and select the wells and positions to be captured, as described in the <u>Setup</u> and Operation Manual.
- 10. Select at least 5 capturing positions to capture for each sample. The more heterogenous the cell distribution, the more captures are needed.
- 11. 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).
- 12. Click *Advanced setup* and check *Multiple destination groups*.
- 13. Make sure *One group per well* is checked.
- 14. Click Save and close.
- 15. Click *Capture*. Go to the *View image tab* and review the images for quality.
- 16. Await the time-lapse capturing to finish.



#### For imaging with a fixed stage

- 1. Replace the standard lid with the appropriate *HoloLid*, following the HoloLid protocol.
- 2. Place the sample on the *HoloMonitor stage* using the appropriate distance plate:

Vessel	Distance plate
Sarstedt TC Dish 35, Standard	# 2
Sarstedt TC Plate 6 Well, Standard, F	None
Sarstedt lumox <sup>®</sup> multiwell, 24 Well	# 1
Sarstedt lumox® multiwell, 96 Well	None

- 3. Go to the *Live capture tab* and ensure that the images are well focused. Adjust software focus, if required.
- 4. Create one *Group* per sample, i.e. "Control1 Day 1" and "Treated Day 1".
- 5. Capture at least 5 images per sample, the more heterogeneous the cell distribution, the more captures are needed.
- 6. Repeat capturing (steps 4-5) 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).

View images	📌 identity cells	% Irack cells	s 🛃 Analyze data 📄 Cell count 🛛 👦 Export images				
			Cell Count Report Number of cells in vessel: 1.17e+006 ±14% Number of cells per ml: 2.34e+005	Area (µm²) distribution		Sebastian JIMT1 Databas Project 13/10/2016 10.30 Sebastian Group	se 📰 🖉 🖉 🖉 🖉 🖉 🖉 se 🖉
			Confluency: <b>29% ±4</b>	137		13/10/2016 10:37         Capture#1           13/10/2016 10:37         Capture#1           13/10/2016 10:37         Capture#1           13/10/2016 10:37         Capture#1           13/10/2016 10:37         Capture#1	October 13 (Well A1) I October 13 (Well A2) I October 13 (Well A3) I October 13 (Well B1) I October 13 (Well B2)
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			Report date: 27/01/2017 15:32 Capture at: 13/10/2016 10:44:01 - 10:46:54	367		Count: 149 Cooff: 34%	Take #1: (94.480; 15.09
			Vessel growth area: 25 cm <sup>2</sup> Vessel media volume: 5 ml	258		Count: 142	Holographic (x20) 13/10/2016 10:44:24
			Total number of images: 15 Total imaged area: 4.016 mm <sup>2</sup> Total number of cells in images: 2471	128- 89- 43-		Count: 168	Holographic (x20) 13/10/2016 10:44:36
			Number of cells on image edge: 438	0 125.7 1.09E3 4.01E3 5.70E3 7.50E3 9.07E3 1.18E4 1.39E4 1.00E4	]	Count: 109	Holographic (x20) 13/10/2016 10:44:48
lessel growth area. lessel media volum	e: 25 cm² ¥ 5 ml ¥	Cell un	t per ml ▼ Area-histogram: ✔ Auto 603 · 2764 μ Volume histogram: ✔ Auto 125.7 · 16027.1 μ	m <sup>®</sup> No. of bins: 45 Save report ♥ Include histograms m <sup>®</sup> No. of bins. 45		Confl: 20%	Take #1: (90.002; 18.0) Holographic (x20) 13/10/2016 10:45:01
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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*.