PHASE HOLOGRAPHIC IMAGING

HoloMonitor® M4

CELL MOTILITY PROTOCOL

MATERIAL

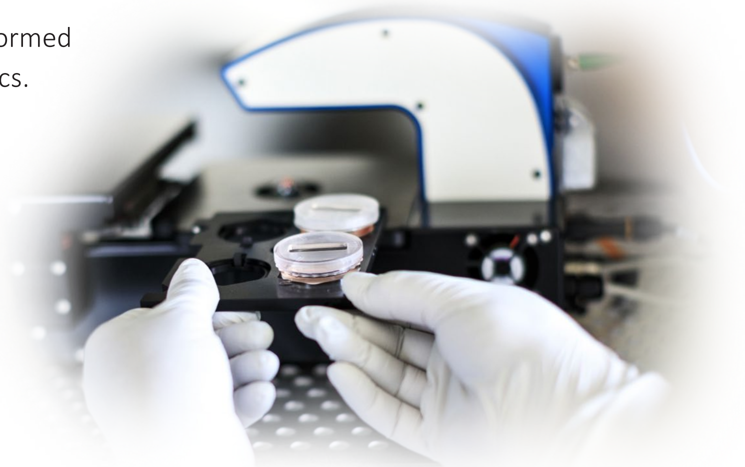
- **HoloMonitor® M4**, placed inside a cell incubator.
- **Hstudio software,** version 2.7.1 or later.
- **Culture vessel** by choice:
  - Sarstedt TC Dish 35, Standard (cat. # 83.3900)
  - Sarstedt TC Plate 6 Well, Standard, F (cat. # 83.3920.005)
  - Sarstedt lumox® multiwell, 24 Well (cat. # 94.6000.014)
  - Sarstedt lumox® 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® multiwell, 24 Well	71130
Sarstedt lumox® 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 support@phiab.se.
- **Cells** suspended to reach a confluence of 2-5 % when seeded (approx. 6 000-11 000 cell/cm^2 for L929, A375, and Jimt-1 cells). Other cell types may require a different seeding concentration.
- Setup and Operation Manual 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.



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PREPARATION

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

Volume	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® multiwell, 24 Well
170 µl/well	Sarstedt lumox® 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.

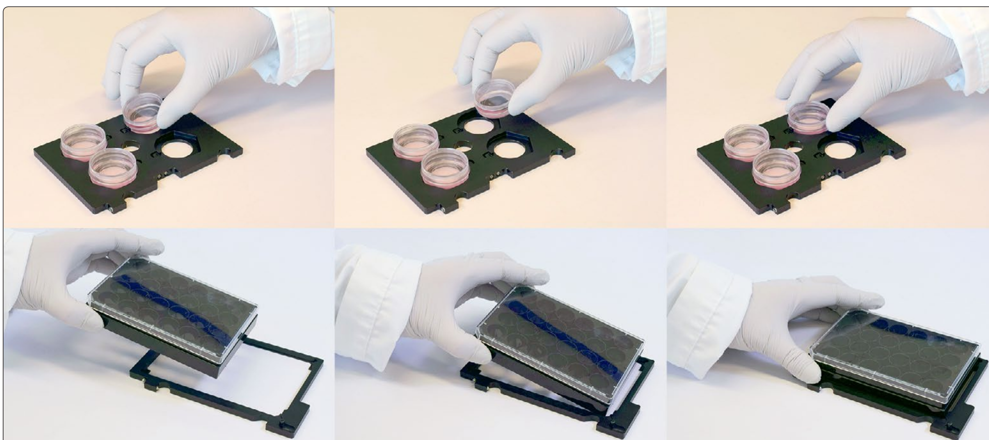
IMAGING

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.

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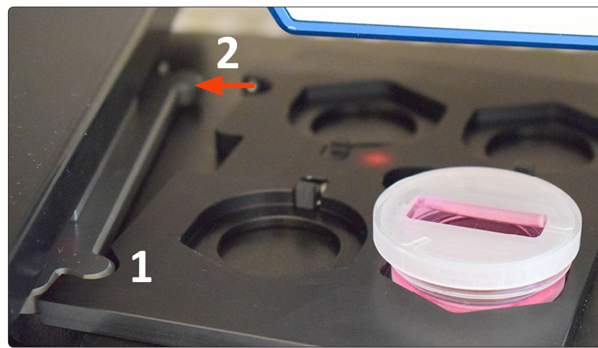
4. Thereafter place the ***Vessel holder*** with the samples on the HoloMonitor stage:



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. 5 minutes between captures is recommended. However, for very fast cells an even shorter interval may be required.

9. Check ***Capture pattern*** and select the wells and positions to be captured, as described in the Setup and Operation Manual. Alternatively select capture positions and click the ***Remember button*** for as many positions as required. However, short interval between image captures (< 5 min) limits the number of positions that can be captured.

10. Click ***Advanced setup*** and check ***Multiple destination groups***.

11. Make sure ***One group per well*** is not checked.

12. Click ***Save and close***.

13. Click ***Capture***.

14. Go to the ***View image tab*** and review the images for quality.

15. Await the time-lapse capturing to finish.

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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® 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 the software focus, if required.

4. Create a ***Group***.

5. Check ***Timelapse*** and type the total time and interval of the time-lapse capture. 5 minutes between captures is recommended. However, for very fast cells an even shorter interval may be required.

6. Click ***Capture***.

7. Go to ***View image*** and review the images for quality.

8. Await the time-lapse capturing to finish.

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ANALYSIS

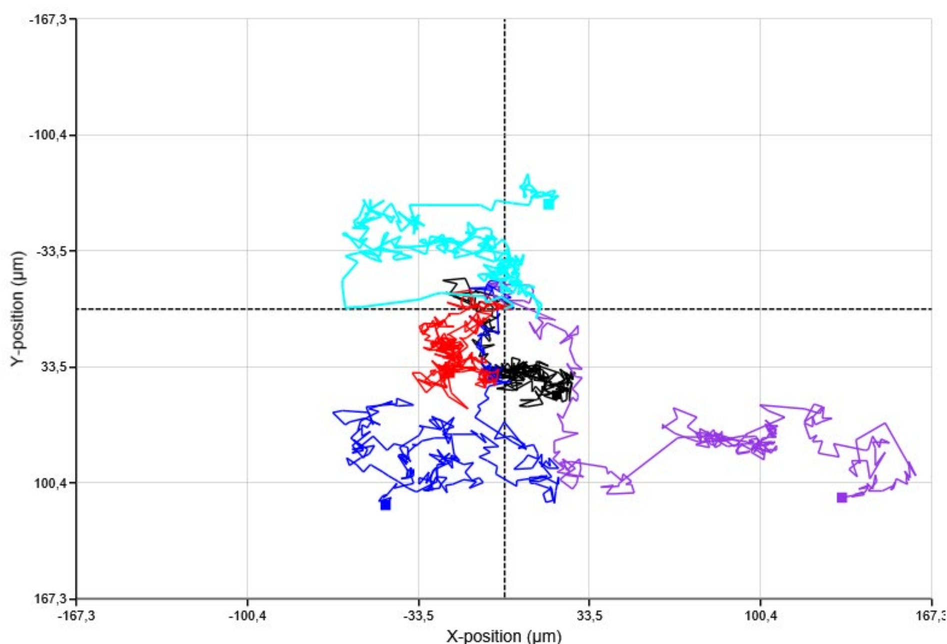
1. Go to the ***Identify cells tab***. Check the segmentation and, if needed, adjust the ***Threshold*** and ***Min object size*** settings to fit the cells for all ***Groups***. The settings can be applied for all images within each ***Group*** but need to be validated and possibly adjusted for all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

2. Go to ***Track cells***. Add all frames from one position to the tracking analysis. Individual cells to be tracked are added by clicking on them.

3. Move the ***Timeline slider*** to the right to see the tracks of the added cells. Adjust the possible errors of the software using the ***Warnings list***.

4. When the cells divide, a warning sign appears in the frame. This indicates a cell division. Click ***Division*** if both daughter cells are to be tracked (optional). Otherwise, a random daughter cell is selected and subsequently tracked by the software.

5. Go to ***Plot movement*** and check all cells to be included in the plot. The colored tracks show the movements of the selected cells, with the origin as the starting point for each cell:



The plot can be saved as an image in several formats. If different samples (treatments) are to be compared it is recommended to adjust the X- and Y-scales to be identical for all samples before export to XML-files.

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6. By hovering over the tracks, quick access to the data for each time point is achieved:

7. Rename the analysis (***Tracking 1 tab***) to reflect the sample e.g. "Control 1".

8. Activate a new tracking tab (***Tracking 2***) and repeat the analysis procedure with image frames from the next capture position. Repeat for as many positions and samples as the experiment comprises.

9. To be able to resume the analysis later, save each sample analysis. Go to the top menu, click ***Tracking*** and then ***Save as***.

10. For further analysis, export the tracking data to an XML-file. Open the XML-file in Excel. In the ***Motility tab***, the values for each cell at the end of the time lapse is at the right most column. ***Motility speed***, ***Migration*** and ***Migration directness*** are also accessible in the spread sheet together with all the parameters (default value) or the selected parameters (***Features to export***):

11. Repeat from step 1 for all samples/***Groups***.

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