HoloMonitor® M4 PROTOCOL

CELL MOTILITY

April 2017, Rev. 1

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 and protocols for their use*** (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.

[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 2-24h.

START-UP THE IMAGING

Day 0-1

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. 5 minutes between captures is recommended.

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

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

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

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

4 For imaging with a fixed stage:

4.1 Create a ***Group***.

4.2 Focus the image using the correct distance plate.

4.3 Click ***Time-lapse*** and type the total time and interval of the time-lapse capture. 5 minutes between captures is recommended.

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

4.5 Wait for the time-lapse capture to finish.

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 ***Frame***s. 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 soft-ware 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 (Figure 1). 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.

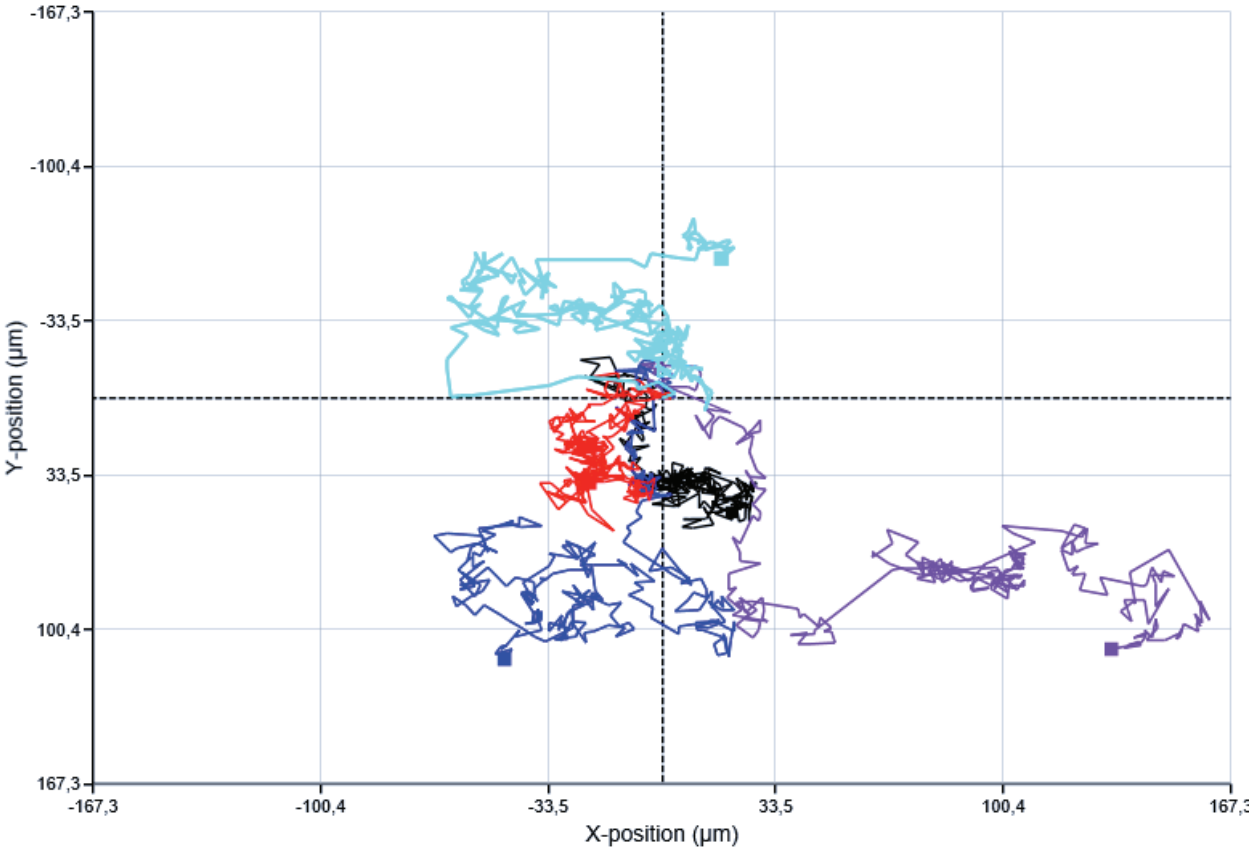

Figure 1. Cell movement tracks.

6 By hovering over the tracks, quick access to the data for each time point is achieved (Figure 2).

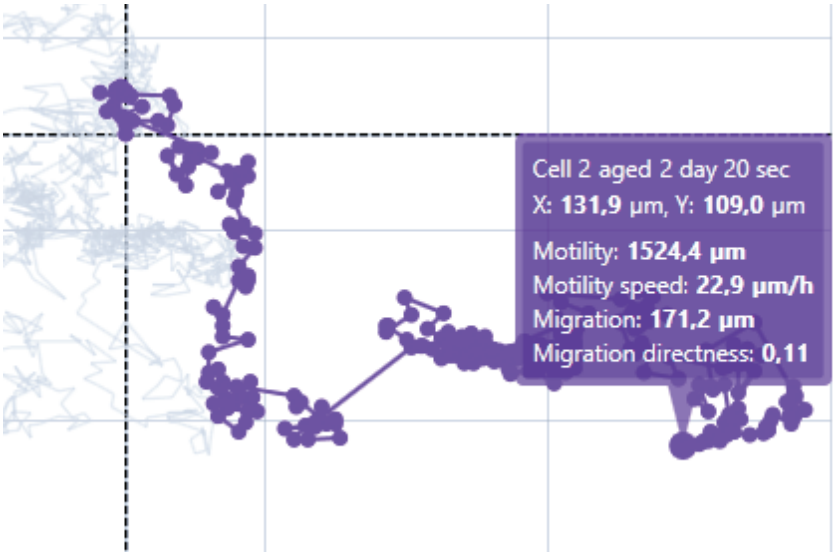

Figure 2. Information for a chosen cell.

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 (Figure 3). ***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***).

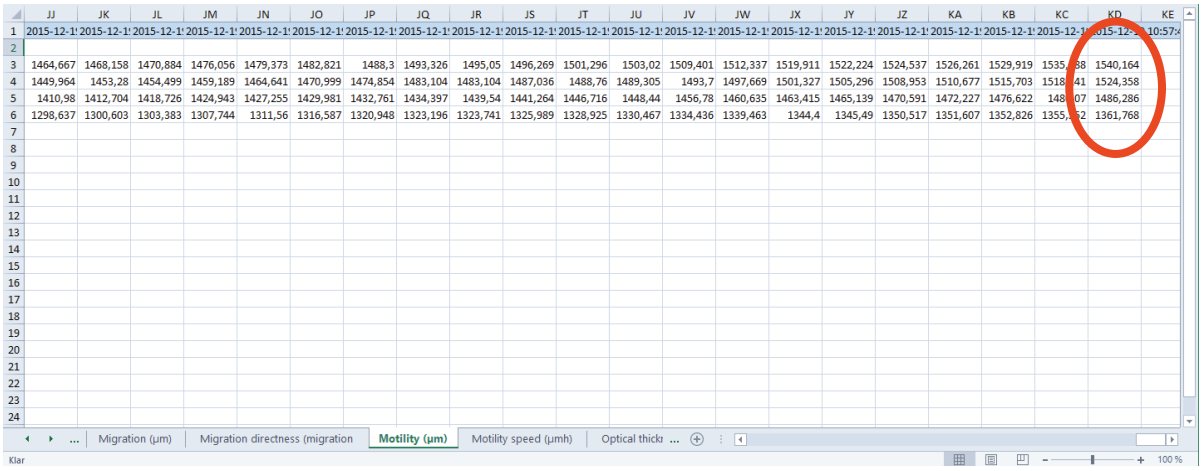

Figure 3. Tracking data presented in a spread sheet.

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