

# HoloMonitor<sup>®</sup> M4 PROTOCOL

## MITOSIS LENGTH

June 2017, rev. 2

### 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), Sarstedt 35 mm cell culture dish (growth area: 8 cm<sup>2</sup>) or Ibidi 1-well  $\mu$ -slides (growth area 2.5 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** ([www.phiab.se/products/hololids](http://www.phiab.se/products/hololids)).
- **Setup and Operational manual** for using HoloMonitor ([www.phiab.se/support](http://www.phiab.se/support)).

<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  $\mu$ l/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

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### 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 **Frames**. Discard bad frames.
- 2 Go to **Track cells**. Add all frames from one position to the tracking analysis. Go through all the frames to manually select each newly rounded cell. Each selected cell acquires individually colored outlines (Figure 1B, red arrows).

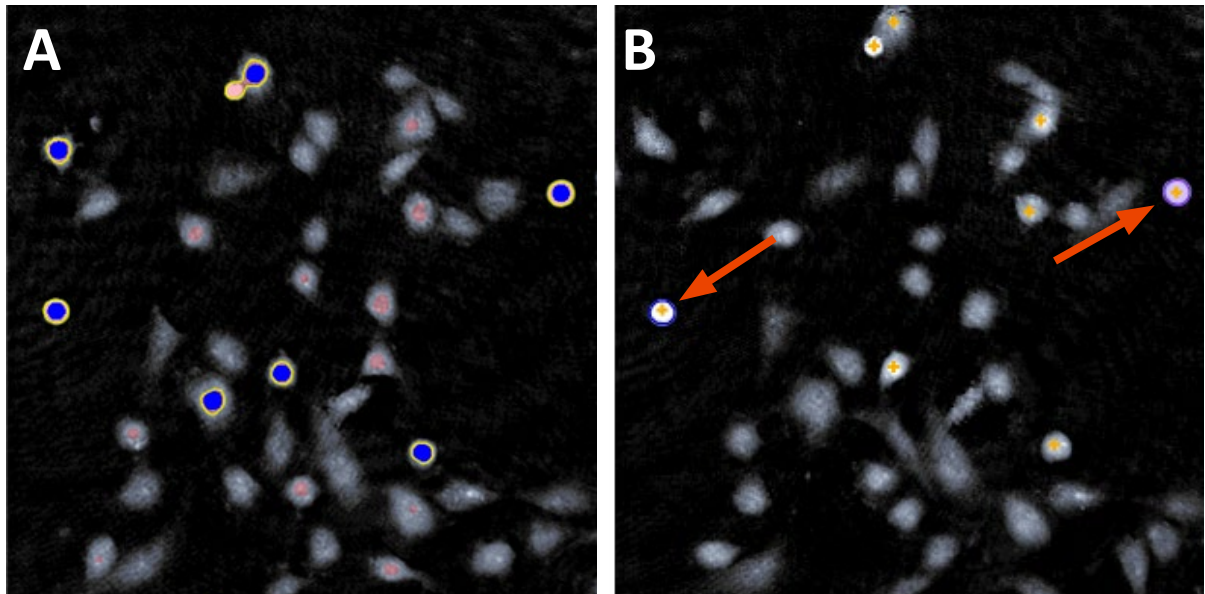


Figure 1. A: Thresholding only rounded up cells. B: Colored outlines indicating selected cells.

- 3 When the cells divide, a warning sign appears in the frame (Figure 2). This indicates a cell division. When the warning appears, it is time to unset that cell. If the cell is covered with the warning sign, zoom in to be able to unset it (otherwise the warning message appears when hovering over it).
- 4 Continue to select and unset cells until the end of the time-lapse.



Figure 2. Cell division warning.

In some cases, e.g. after asymmetrical cell divisions, the software loses the cell and the tracking jumps to the closest suitable cell (Figure. 3). The user must select that cell and then unset it.

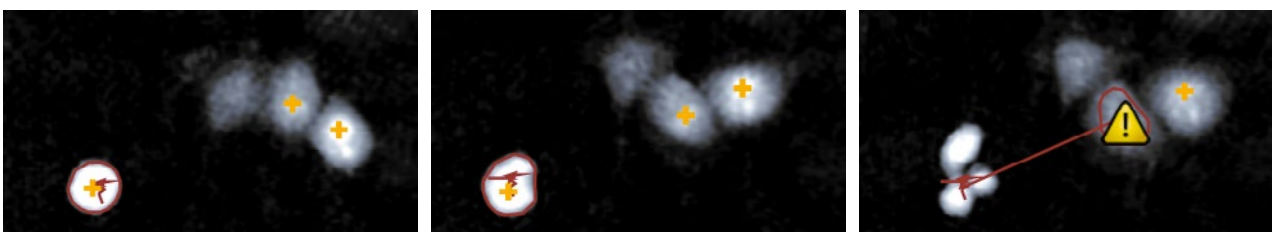


Figure 3. Imperfectly tracked cell after asymmetric division.

## RESULTS

- 1 Click **Plot features**. A graph showing the length of the mitosis for each cell over time appears (Figure 4). Check each box to display all individual cells. Each colored line corresponds to one mitotic cell. Set the Y-axis parameter to **Center position x**. The x-axis denotes the time. Each time-point corresponds to a captured frame. In the example below, some cells stay in mitosis only for one frame, and can be seen as a single marker. The longest mitosis lasts until the end of the movie, and is seen as a long string of markers. That cell is stuck in mitosis. If the line bends, it means that the cell has changed position in the image frame (i.e. a change of Center position). This diagram is a highly visual way to see the data.

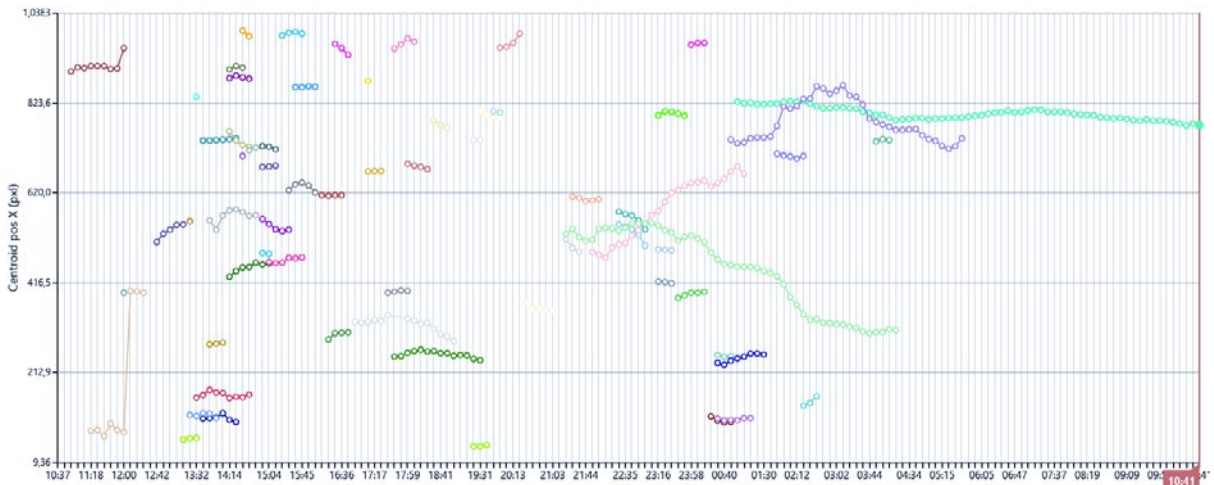


Figure 4. Cell movement displayed as tracks.

- 2 To be able to recall the analysis later, save each sample analysis. Go to the top menu, click **Tracking** and then **Save As**.
- 3 For further analysis, export the tracking data to an XML-file. Open the file in **Excel**. In the first worksheet you will see the number of tracked cells and the number of frames (Figure 5, left). Each tracked cell has its own tab (see bottom of calculation sheet).

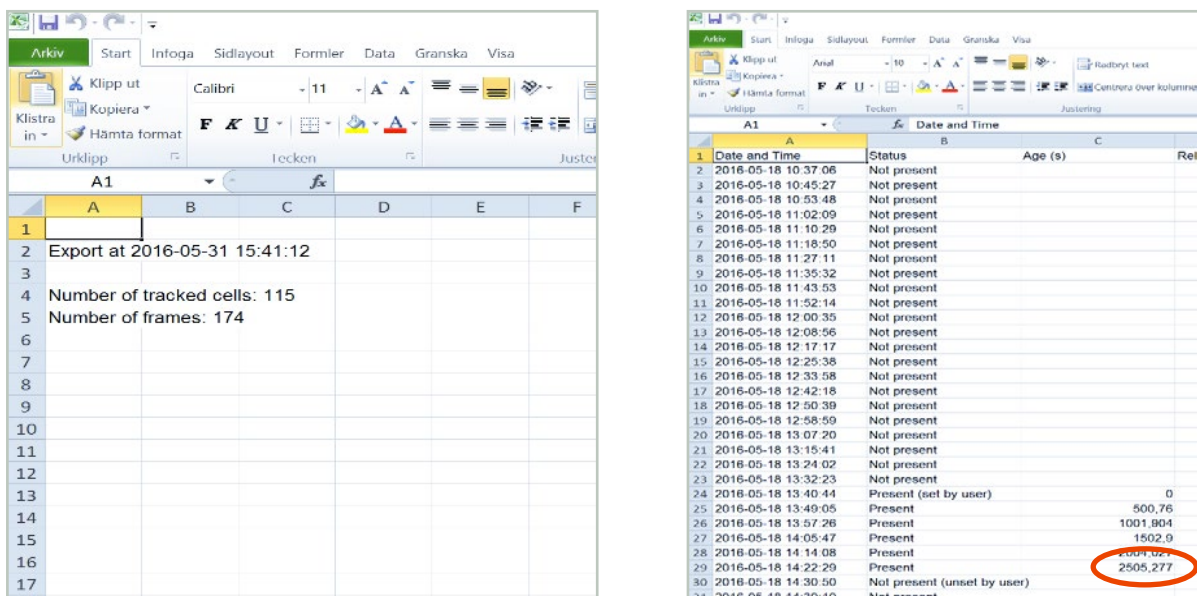


Figure 5. Mitosis length data presented in a spreadsheet.

- 4 Click the individual cell tabs (Figure 5, right). You will see in which frames the cells were in mitosis and for how long (in seconds) the cells were in mitosis by the numbers found in the “Age” column, or the column C (red circle). **The total mitosis time** is the last and largest number in the column, as the numbers for each frame is added to the previous frame from the onset of the cell. If the cell status is denoted **Not Present**, the cell is not in mitosis and not selected by the user. **Present** denotes that the cell is selected and therefore in mitosis.
- 5 To collect the mitosis length of all cells, create a new tab in **Excel**.
- 6 Type **Cell 1** in A1 in the tab you just created.
- 7 Use the small rectangle in the bottom-right corner of the cell and expand it to the right for as many cells as there are cell tabs in the exported file.
- 8 Add the formula = **MAX(INDIRECT(“”&A1&””!C2:C175”))** to A2. The interval which is exemplified here with **C2:C175**, is meant to include all time points of your experiment. You might have to extend the interval C2:C175 to cover all time points in your experiment, i.e. type the correct last number for column C (instead of C175 as in this example).
- 9 Use the small rectangle in the bottom-right corner of the cell again, but now in the cell where you just typed the formula. Expand it to the same length as in step 6 (Excel sometimes needs saving, closing and re-opening to make this function work).