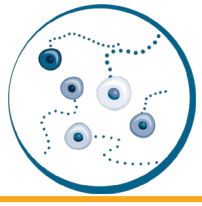
HOLOMONITOR® APP SUITE

Pre–Study, Kinetic Motility Assay Protocol



For optimal outcome of Kinetic Motility studies using HoloMonitor® App Suite, the settings for duration, interval and number of positions needs to be selected carefully. This protocol shows how to calculate these parameters prior to setting up a motility study.

Please note that this procedure is based on the Kinetic Motility Assay, thus you will need a ***Kinetic Motility Assay Protocol.***

BACKGROUND

When using HoloMonitor for cell motility studies it is vital that the capture settings are optimized for each cell line. There are three parameters to consider: ***duration***, ***interval*** and ***number of positions***. Fast-moving cells need to be captured frequently in order to acquire a correct velocity estimate. They may also move out of the field of view. Therefore, fast-moving cells should be captured as often as every 1-5 minutes. Slow-moving cells can be captured every 6-20 minutes. As a guideline: between captures cell should have moved a distance that is maximum 40% of its diameter (1).

Too frequent capturing however, limits the ***number of positions*** that can be included in the experiments due to speed limitation of the HoloMonitor stage. On the other hand, at least 40 cells should be included in the experiment from the start, to get reliable statistics (1). The App Suite software calculates the time needed for the chosen settings and warns when the limitations are exceeded.

Duration must also be selected carefully. The duration should allow the cells to move on average 10 times the cell diameter, i.e. 1-2 hours for fast-moving cells and 12-36 hours for slow-moving cells. However, the more confluent the culture gets, the less will cells move, and confluent cultures provide no additional information about motility, but introduces artefacts instead. In addition, the longer the duration, the more images and hence datafiles is produced and stored. Due to the large amount of data acquired, the duration of the capturing should be a short as possible. To be able to setup an experiment, when the cell velocity is unknown, a 4-hour pre-study on untreated control cell is recommended.

In case the cell diameter and velocity are known, go directly to the Calculation section.

CELL PREPARATION AND INSTALLATION OF THE HOLOMONITOR INSTRUMENT

1. Follow all steps in the ***Kinetic Motility Assay Protocol***.

BASIC SETUP

1. Follow all steps in the ***Kinetic Motility Assay Protocol*** but select only one well.

CAPTURE SETUP

1. Follow the ***Kinetic Motility Assay Protocol***. Set the ***Duration*** to 4 hours and ***Frequency*** to one image per 5 minutes. Set ***Positions per well*** to 1 in the selected well. In this step, duration and interval of the experiment are set. Capture positions for imaging are selected and the image quality can be validated.
2. Click Settings (red arrow, fig. 1) and uncheck the box ***Move to resting position between time points*** (green arrow, fig. 1).

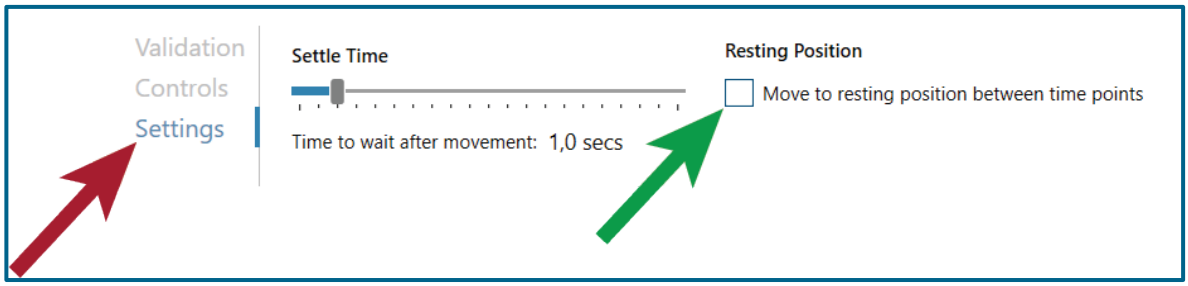


Figure 1. Ensure that the checkbox (indicated by green arrow) ***Move to resting position between time points*** is deselected.

3. Ensure that the selected ***one position*** is well focused. To move the stage there, click on the small square in the selected well (depicting the position) in the well. Focus is adjusted in the Control tab, see the ***Setup and Operational Manual*** for details.
4. Start the capturing and close the experiment tab when finished, as described in the ***Kinetic Motility Assay Protocol***.

ANALYSIS

1. Follow the ***Kinetic Motility Assay Protocol*** until the graphs are drawn (fig.18 in ***Kinetic Motility Assay Protocol***). In the graph showing accumulated mean distance (bottom graph in fig. 18), the distance covered after 4 hours is seen by hovering the mouse pointer over the last data point on the time scale (X-axis, black balloon in fig. 2, lower graph). From this value mean speed (µm/min) can be calculated as described in the ***Calculations box*** below.

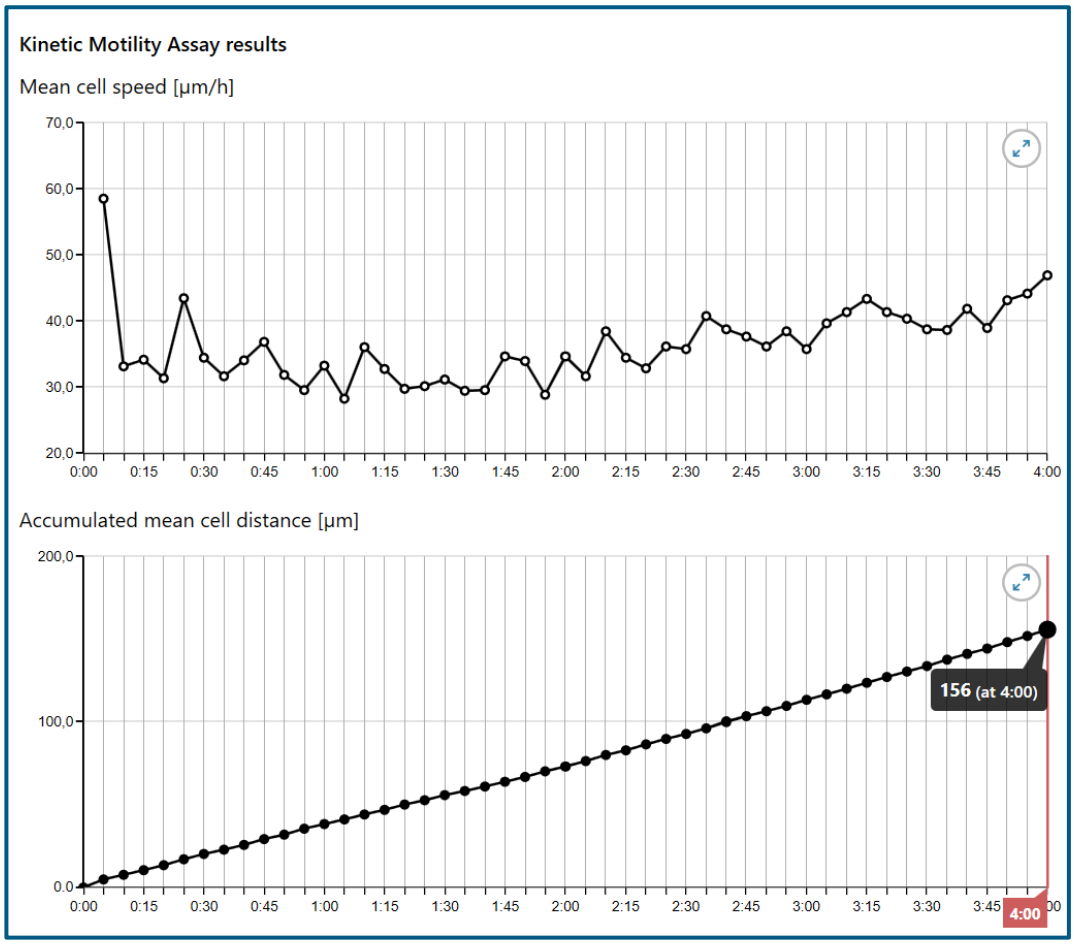


Figure 2. The ***Kinetic Motility Assay*** results page. Black balloon shows the distance cells have accumulated in 4 hours, in this case: 156 µm.

Calculations for Optimal Capture Interval

The optimal capture interval is defined as the time it takes for the cells to move about 40% of its diameter, hence:

$$Optimal\ capture\ interval = \frac{0.4 \times \text{cell diameter}}{\text{cell velocity}}$$

where the average cell diameter can be determined using the Cell QC application and the cell velocity is calculated from the accumulated distance graph as

$$Cell\ velocity = \frac{\text{mean distance}}{\text{duration}}$$

Duration

The *minimum duration* equals the time it takes for the cells to move 10 times its diameter:

$$Minimum\ duration = \frac{10 \times \text{cell diameter}}{\text{cell velocity}}$$

The *maximum duration*, defined as the time point when the confluence reaches 70%.

Number of positions

At least as many as needed to include 40 cells/treatment.

Example

If the cells has moved 159 µm in 240 minutes the *cell velocity* equals 159 µm/240 min = 0.662 µm/min. Hence, for an average *cell diameter* of 22.6 µm, the *optimal capture interval* is 0.4 × 22.6 / 0.662 = 13.7 minutes. The *minimum duration* is 10 × 22.6 / 0.662 = 341 minutes or 5h 41 min.

Note that depending on time for treatment to take effect, the duration may have to be extended. Just make sure that the confluence does not exceed 70%.

REFERENCE

1. Roman Zantl and Elias Horn (2011) Chemotaxis of slow migrating mammalian cells analyzed by video microscopy, in Cell migration, developmental methods and protocols. Claire M. Wells and Maddy Parsons (Ed.) ISBN: 978-1-61779 206-9, Humana Press c/o Springer Science, New York, USA