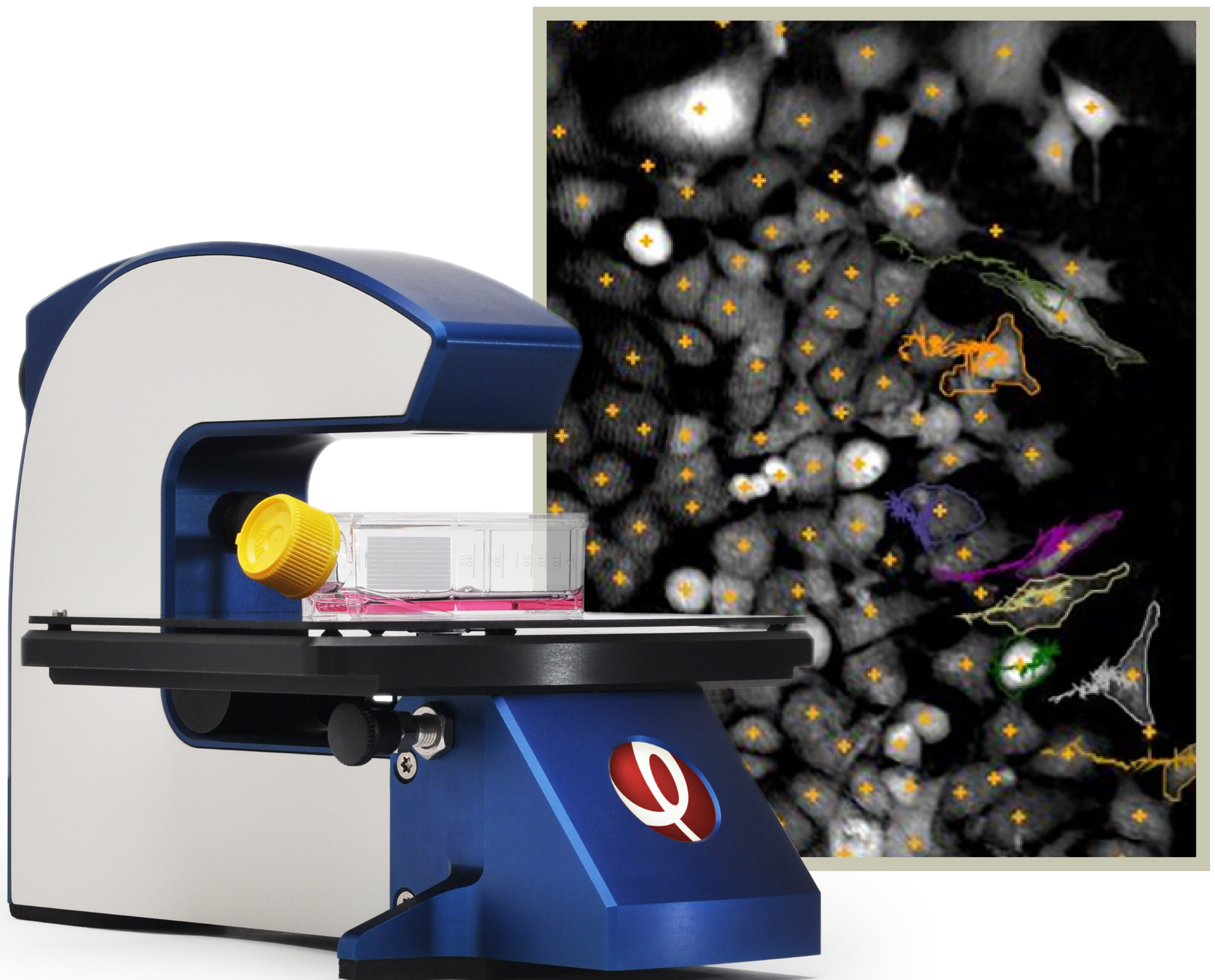
HoloMonitor

application note on

Label-free Cell Motility



Holographic Imaging
Phase

Label-free Cell Motility

Understanding the movement of cultured cells improves the understanding of cell movement and signaling in living organisms. The HoloMonitor® time-lapse cytometer offers unambiguous, non-invasive quantification of cell migration and motility.

BACKGROUND

Cell movement is categorized by non-directional cell motility speed and by directional migration velocity (Entschladen et al. 2005). Motility is regarded as random cell movement occurring in almost every cell culture while migration is a response to a cell attractant or repellent.

HoloMonitor has been used by Persson et al. (2013) for live cell imaging and tracking of fibroblasts, cultured on nanowire arrays. The motility of the cells decreased when growing on nanowires compared to growing on normal polystyrene plastics. HoloMonitor was also used to study the motility of breast cancer stem cells treated with a drug candidate (Cirenajwis et al. 2010). The drug candidate affected the motility of stem cells specifically. HoloMonitor was the instrument of choice as cells can be long term analyzed in normal cell growth media and environment, with no labeling, staining or phototoxicity.

In this application note, we illustrate how HoloMonitor can be used to quantify and distinguish between cell migration and motility. Movement of individual cells was quantified at room temp and at 37˚ C, and scratch assay used to distinguish between migration and motility.

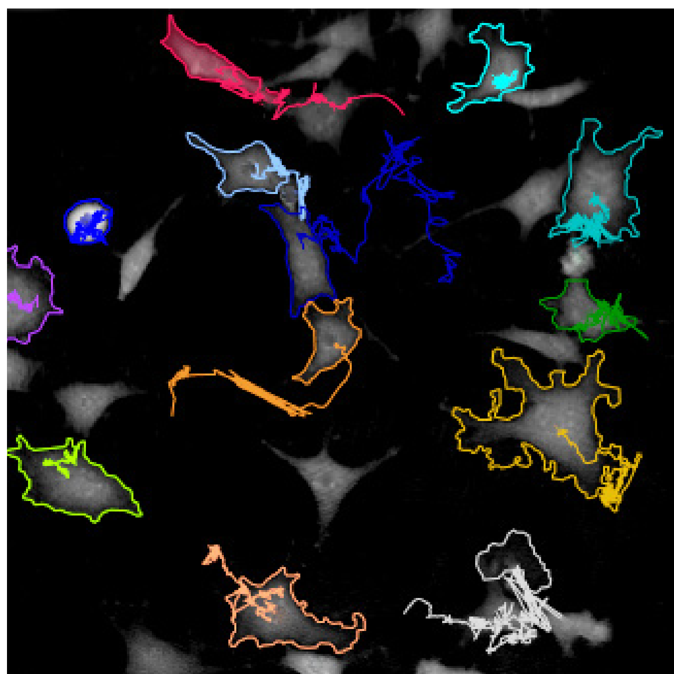
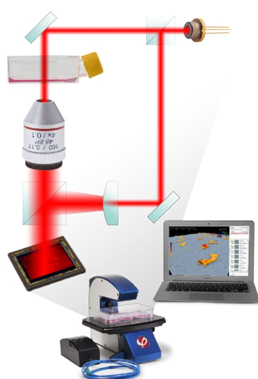


Figure 1. *A HoloMonitor image with movement tracks of mouse fibroblasts over 24 hours at 37˚ C.*

HOLOGRAPHIC MICROSCOPY

HoloMonitor create label-free images by dividing red laser light into a reference and an object beam (right). As the object beam passes through the specimen, a phase delay is imprinted on the beam. By subsequently merging the object and the reference beam, this otherwise invisible imprint is recorded by an image sensor. From the recorded hologram, the imprint is numerically reconstructed into a so-called phase image, which is displayed and analyzed (Mölder et al 2008).



METHODS

Temperature dependent motility

L929 mouse fibroblast cells in plateau phase were sparsely seeded and allowed to adhere for 24 hours. Thereafter they were monitored with or without a heating plate (Ibidi) set to 37° C. One position in each cell sample was selected to be captured every four minutes for 24 hours. In each time-lapse sequence, 16 cells were selected to be tracked. HoloMonitor analysis tracks the movements of individual cells. Such tracks may either be viewed directly in cell images (Fig. 1) or as spatial plots (Fig. 2). When tracking a cell, the software automatically measures individual cell migration and motility.

L929 cells were seeded in 5 cm petri dishes and allowed to grow to confluence. A scratch was created through the mono layer using a dentist utensil. Thereafter the time lapse image capture started.

RESULTS & DISCUSSION

Conventional assays are limited in that they only measure cell population movement. These assays require extensive control samples to avoid that random cell movement is interpreted as cell migration. The popular Boyden assay is intrinsically ambiguous as it measures the sum of cell migration and motility. When using HoloMonitor for motility and migration analysis, not only the speed and direction of movement is obtained, but there is also the opportunity to determine whether there is a true directional migration of a cell population or a random movement.

Temperature dependent motility

Cell movement is dramatically greater at 37° C than at room temperature (Fig. 2). This is confirmed by the measured average cell migration and motility values (Table 1). In the HoloMonitor analysis tool, motility is defined as the actual path of the cell while migration is the shortest distance between start- and endpoint. Directness is the ratio of migration and motility, indicating how direct a cell or a cell population moves. Random movement has a directness of 0. A higher value indicates a direction in the overall movement. Movement along a straight line gives a directness of one.

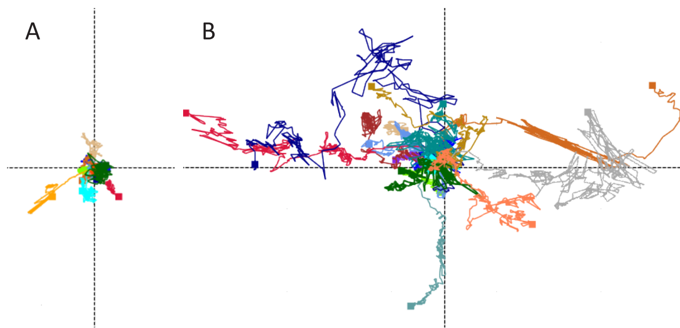


Figure 2. *The movements of mouse fibroblast during 24 hours at room temperature (A) and at 37° C (B) presented as spatial movement graphs where all cells start at origin. Each colored line represents the actual path of one cell along the X and Y-axis.*

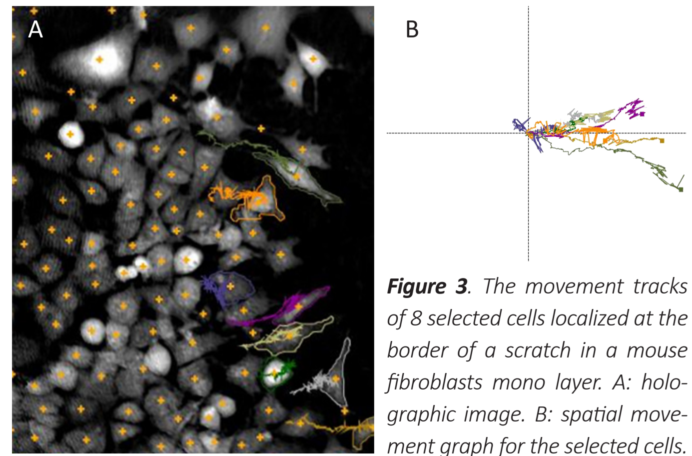


Figure 3*. The movement tracks of 8 selected cells localized at the border of a scratch in a mouse fibroblasts mono layer. A: holographic image. B: spatial movement graph for the selected cells.*

Table 1*. Migration, motility and directness for cells grown at room temperature and 37° C. The values represent the average for 15 cells.*

	Migration (µm)	Motility (µm)	Directness
Room	5	150	0.03
37° C	40	790	0.05

Table 2. *Migration, motility and directness for cells in a scratch assay. The values represent the average for 5 cells.*

	Migration (µm)	Motility (µm)	Directness
Cells at border	42	280	0.15
Cells in crowd	18	290	0.06

Although individual cells move longer distances at 37° C than at room temperature it is not an indication of cell population migration, as the individual cell tracks move in all directions (Fig. 2). The directness value is low (Table 1), also indicating random movement rather than migration. Here we show that the speed of motility is 4.5-fold faster for cells grown at 37° C than at room temp. This is well comparable to the 4-fold difference found by others (Hartmann-Petersson et al., 2000), showing that HoloMonitor time-lapse imaging is a competent tool for motility and migration studies.

Scratch assay

Cells at the border of the scratch in the mono layer moved towards the scratch center (Fig. 3), while cells surrounded by other cells moved randomly (not shown). The migration of cells at the border was 2.3 times higher and the directness was 2.5 times higher than for cells surrounded by cells. Motility was equal for both groups. The higher migration- and directness values for cells at the border (Table 2) compared to cells surrounded by cells, together with the spatial movement graph, show that all cells move in the same direction. This is an indication of a cell population migration (Fig. 3).

When using HoloMonitor time-lapse imaging for scratch assay analysis, it is possible to follow the movement of individual cells and to determine whether the scratch is filled by cell migration or cell division. In other commonly used scratch assay analyses, cell images are captured every 12 or 24 hours, giving no indications of how the scratch is filled.

CONCLUSION

HoloMonitor is very well suited for motility and migration studies, both for individual cells and cell populations. Detailed information is available regarding cell movement, and cells can be monitored for long time periods as there is no labeling, staining or phototoxicity.

REFERENCES

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