

A 3D topographic map showing cell culture structures. The map uses a color scale from blue (low) to yellow (high) to represent the height of the cells. Several distinct, rounded structures are visible, some with a central peak. The background is a light blue, textured surface representing the cell monolayer.

Application Note

Exploiting the potential of HoloMonitor® digital holographic microscopy by combining it with 3D matrix cell culture assays

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ABSTRACT

Culturing cells in a 3D scaffold provide the cells with an environment closer to actual physiological conditions than the more common 2D cell cultures. This is particularly important when studying cell movements as the higher complexity of the microenvironment affect the biomechanics. However, using gels to create the 3D environment usually causes problems for live cell microscopy since cells are found at different focal planes, whereas in 2D, all cells are in the same focal plane. Additionally, the gels themselves may disturb the imaging. Here, a different type of microscope, HoloMonitor M4, based on digital holography, has demonstrated high compatibility with 3D Matrigel cell cultures. We were able to quantify wound closure and cell movement in Matrigel by single cell tracking. We also found possibilities for further applications, such as analysis of drug response, tumor growth, metastasis, and invasion, in our 3D preparations.



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By combining two powerful techniques, digital holographic microscopy using HoloMonitor M4 + 3D matrix embedded cells, we added a third dimension to our cancer cell research. This methodical advance enabled us trap & track cells in the matrix and thereby reveal morphological differences between migratory and invasive mode of cancer cells at the “leading edge”.

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Background

It has been recognized that the cell microenvironment is of the highest importance in cancer research. Since 3D cultures mimic the physiological conditions more accurately than the more common 2D cell cultures, it is essential to develop methods to study 3D cultures. Matrigel mimics the basement membrane with natural proteins of the extracellular matrix. Proteolysis, integrin expression, and the formation of invading structures are some of the cellular behaviors observed during cell migration through the Matrigel matrix. The present study aimed to evaluate the use of HoloMonitor for studies of cell movements and morphology of cells grown in Matrigel 3D environment.

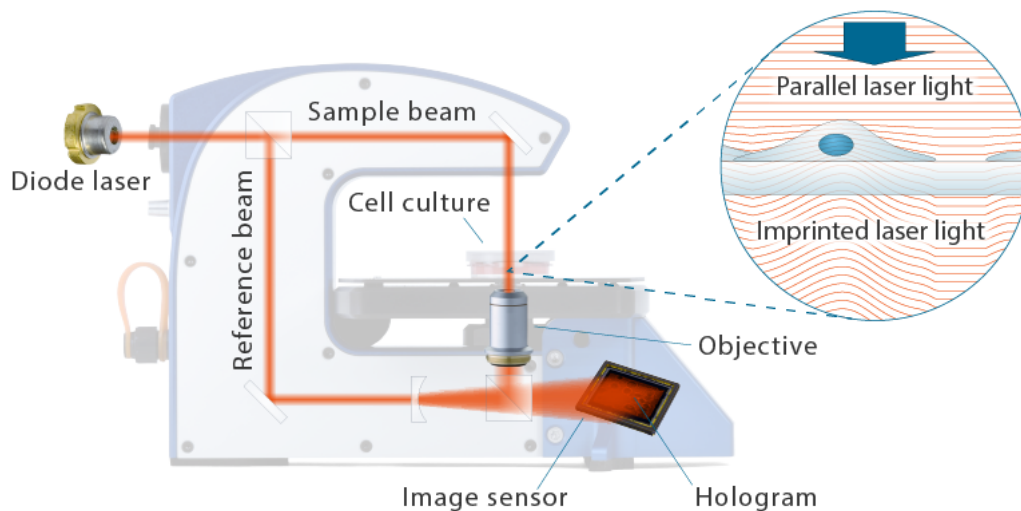


Figure 1. HoloMonitor creates label-free images by dividing red laser light into a reference and an object beam. As the object beam passes through the specimen, a phase delay is imprinted on the beam. After subsequently merging the object and the reference beam, this otherwise invisible imprint is recorded 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

All cell culturing and all preparation procedures are outlined in Hellesvik et al (2020). Three different preparations with or without Matrigel were set up: no Matrigel (uncoated), cells in 1% Matrigel, and cells in 1% Matrigel which was then covered with 50% Matrigel. The overall workflow of wound healing assays is shown in fig. 2.

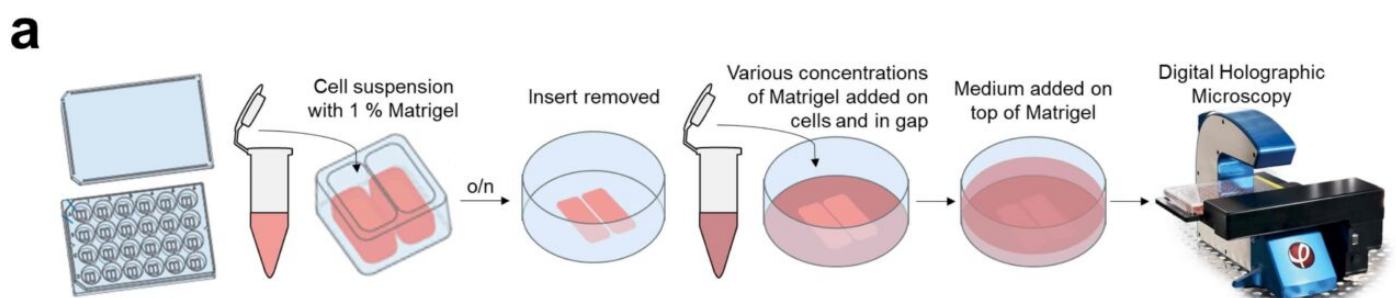


Figure 2. 3D cell culture preparation method for wound invasion assay using HoloMonitor M4

Results and discussion

Various preparations with or without Matrigel were explored for its compatibility with the HoloMonitor system. We found that imaging quality was not notably affected by Matrigel (fig. 3a and b). Cell spreading and irregularity increased in the preparation with 1% Matrigel (fig. 3, c-e). Next, cell proliferation was analyzed using the HoloMonitor. As seen in fig. 3f, there is a slight increase in cell number for 1% Matrigel and a decrease for the 50% Matrigel samples compared to samples with no Matrigel.

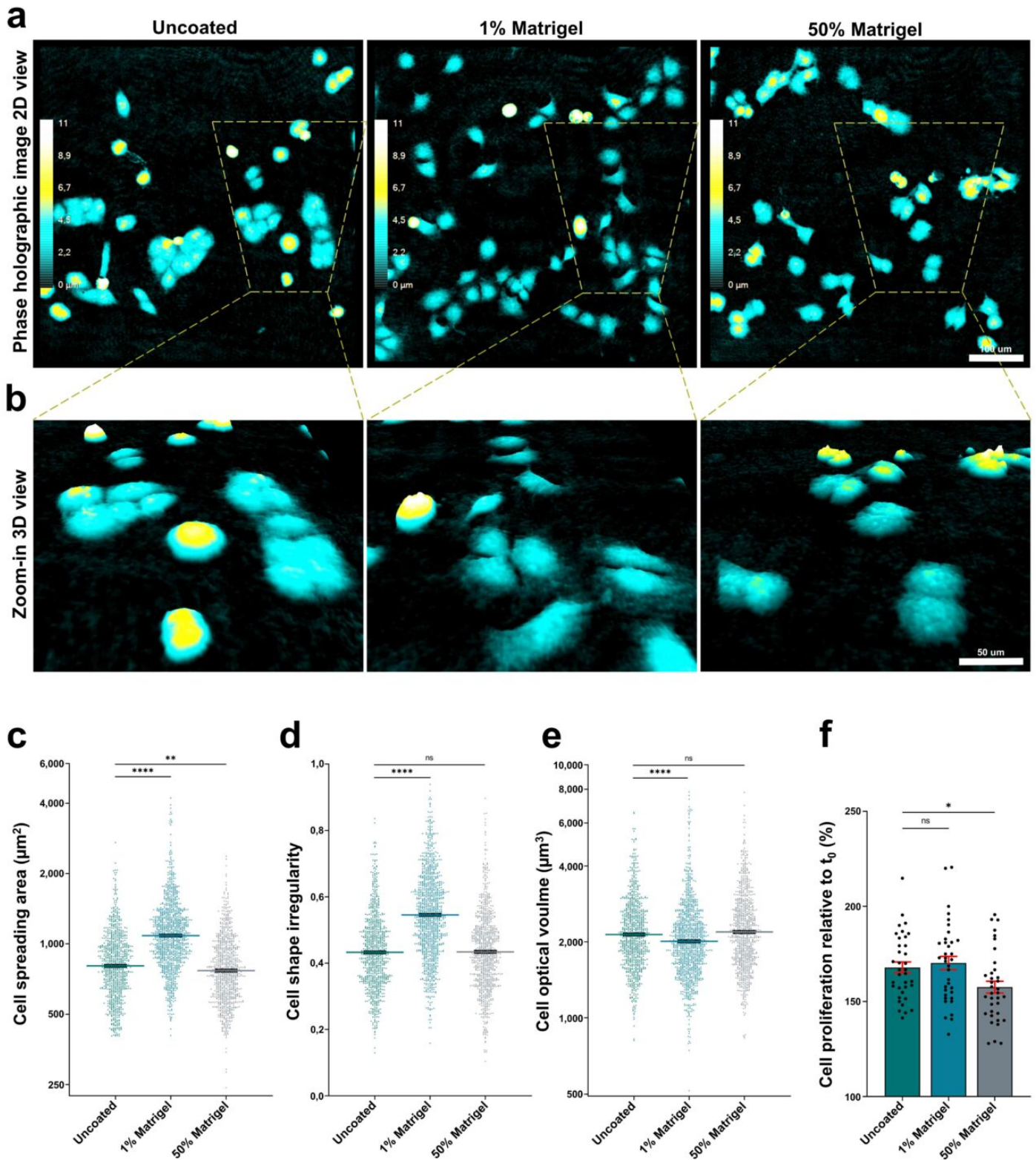


Figure 3. **a**: Representative images by HoloMonitor of uncoated, 1% – and 50% Matrigel, respectively. **b**: close-ups of images in **a**. **c-e**: quantifications of cell spread area, cell irregularity, and optical volume. **f**: cell proliferation relative to t_0 . Shown is the mean \pm SEM of three independent experiments, pooled together. * $p \leq 0.05$, one-way ANOVA with Dunnett's multiple comparison test.

The rose plots showing individual cell tracking in fig. 4a-c demonstrate that in 1% Matrigel, cell movements increased substantially. The same applied for all cell movement parameters, like motility speed, accumulated cell motility, and cell migration in the same preparation (fig. 4 d-f).

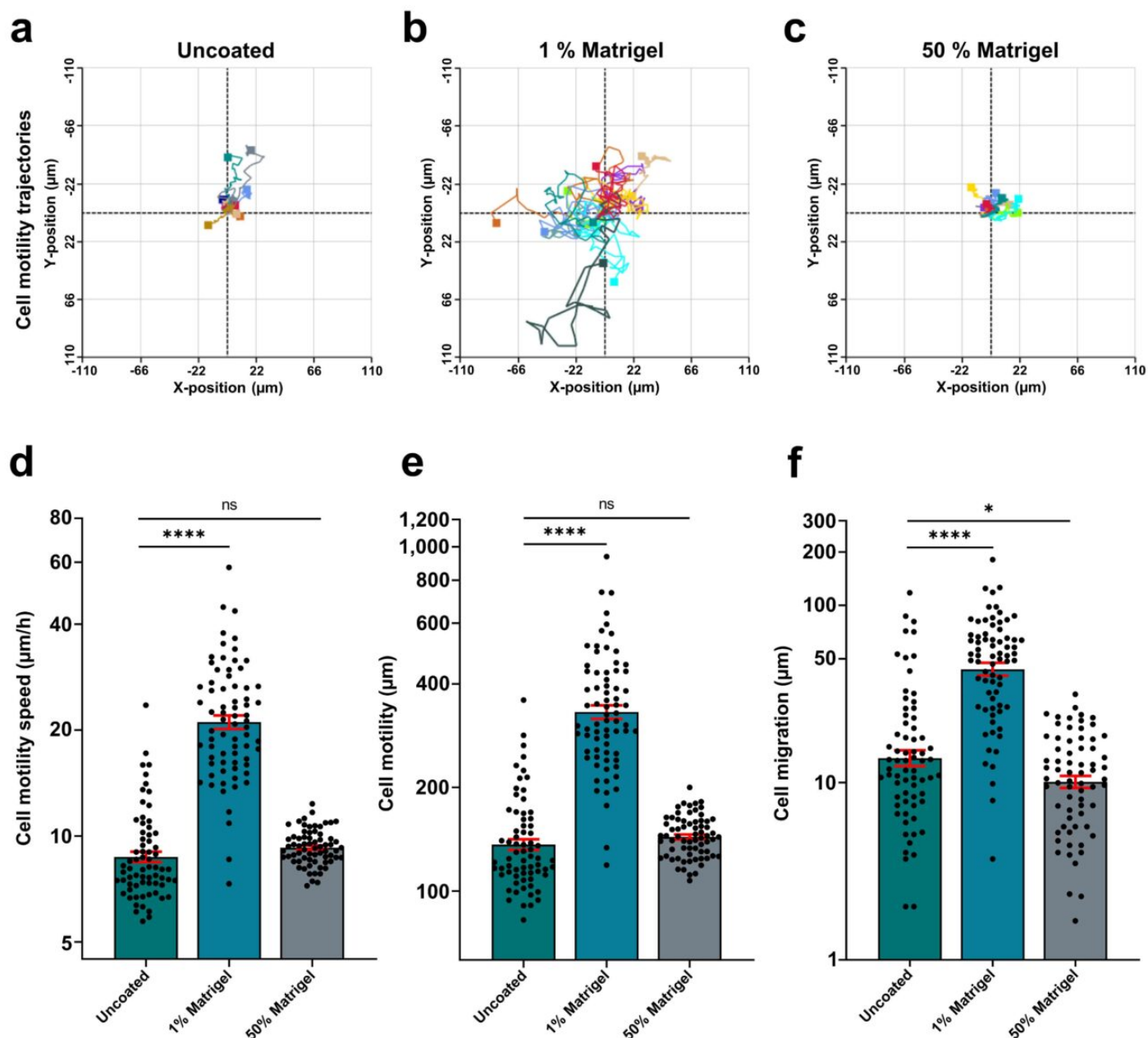


Figure 4. a-c: Rose plots showing single cell tracking for 16h of uncoated, 1%, or 50 % Matrigel substrates, respectively. d-f: cell motility speed, cell motility distance, and cell migration respectively received from the same single cell tracking as in a. Shown is the mean \pm SEM of three independent experiments pooled together. * $p \leq 0.05$, **** $p \leq 0.0001$, one-way ANOVA with Dunnet's multiple comparison test.

An invasion assay was set up using ibidi wound healing inserts (fig. 2). The gap between two areas with densely packed cells was filled with 50% Matrigel. The results were compared to a standard monolayer ibidi wound healing assay. The Matrigel did not reduce the image quality (fig. 5e). In the invasion assay, the Matrigel covered gap (fig. 5, invasion) was clearly closing more slowly than the gap in the standard monolayer ibidi wound healing assay (fig. 5, migration). In addition, the preparation of suspension cells embedded in Matrigel was studied and the growth of smaller cell clusters was quantified together with cells migrating in and out of the cluster (not shown²).

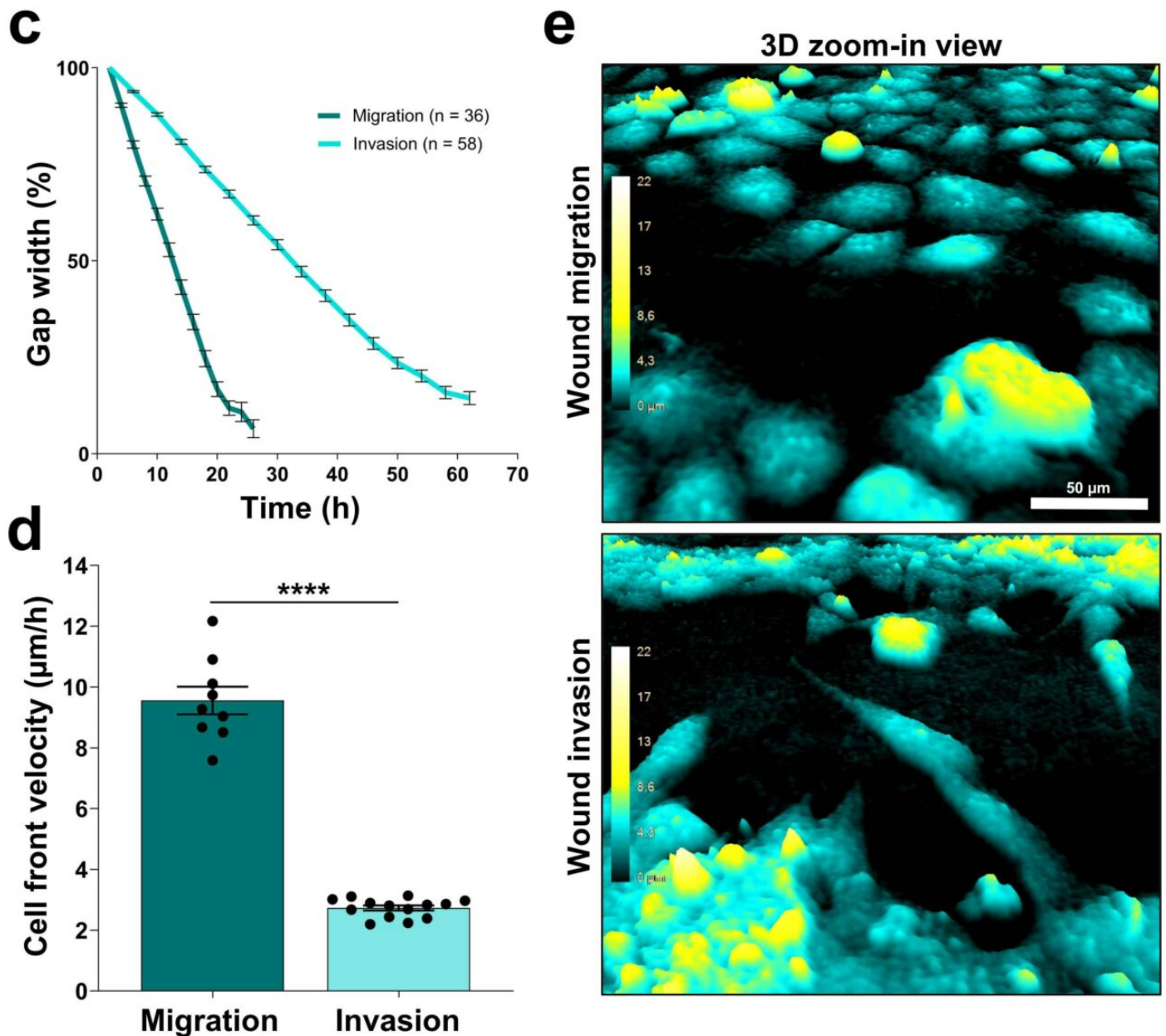


Figure 5. c-e: Gap closure parameters (gap width and cell front velocity) e: Zoomed in tilted holographic images of cell fronts of wound migration and wound invasion respectively used for calculation of the values in c-d. The data are mean \pm SEM from the average of three/five fields of view per well in three replicate wells per experiment and three independent experiments pooled together with $n=9$ / $n=14$ in total for migration and invasion, respectively.

Conclusions

The HoloMonitor M4 was well compatible with the Matrigel preparations used here. There was no difference in image quality or analysis parameters even for the thickest (50-70%) Matrigel preparations when analyzing wound healing or cell invasion. Matrix-embedded suspension cells could also be investigated using the HoloMonitor by quantifying the growth of cell clumps and cells moving out of cell clumps. This study clarifies for the first time that HoloMonitor can be used for monolayer cell cultures embedded in a 3D matrix to study invasion.

References

Mölder et al. (2008), Non-invasive, label-free cell counting and quantitative analysis of adherent cells using digital holography, J. Microscopy, 232:240-247,
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