Imaging living cells without compromising cell integrity

Label-free live cell imaging and analysis for cell population studies by tracking and quantifying individual cells over time — a technical explanation.

The HoloMonitor® label-free live cell imaging system is based on the principle of quantitative phase imaging, enabling non-invasive visualization and quantification of living cells without compromising cell integrity. Here we describe the rationale and advantages of using quantitative phase imaging for live cell kinetic analysis of cellular events — explaining the power of live cell time-lapse cytometry and how cells are made visible without labels or stains.

Peter Egelberg
CEO & Founder, Phase Holographic Imaging

LABEL-FREE LIVE CELL IMAGING — HOW DOES IT WORK?

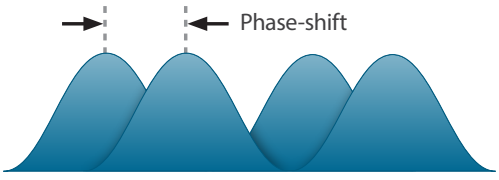
Just like water waves, light waves of a specific wavelength have two principal characteristics: amplitude and phase. Amplitude corresponds to light intensity and is the height of the wave, measured from crest to trough. Phase describes whether a wave is currently at its crest, in its trough, or somewhere in between.

Invisible cells

For a living cell to be visible to the naked eye or in a light microscope, the light arriving from the cell must differ in intensity from the background, i.e. differ in amplitude. Unfortunately, living cells are as translucent as ice cubes in water. They neither absorb, emit nor scatter light to any significant extent and therefore only slightly change the amplitude of the illuminating light, if at all.

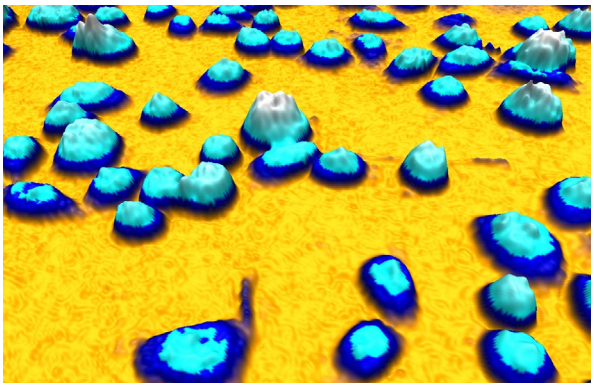
The live cell imaging problem

To be visible in a light microscope, cells must be stained to absorb, emit or scatter light. Unstained living cells do, however, slow down and distort the light passing through them, just like beach waves are distorted by shallower water (above). By using a phase contrast microscope these distortions or *phase-shifts* can be observed, making unstained cells clearly visible. However, conventional live cell imaging using phase contrast microscopy cannot quantify phase-shifts, only visualize them.



THE SOLUTION

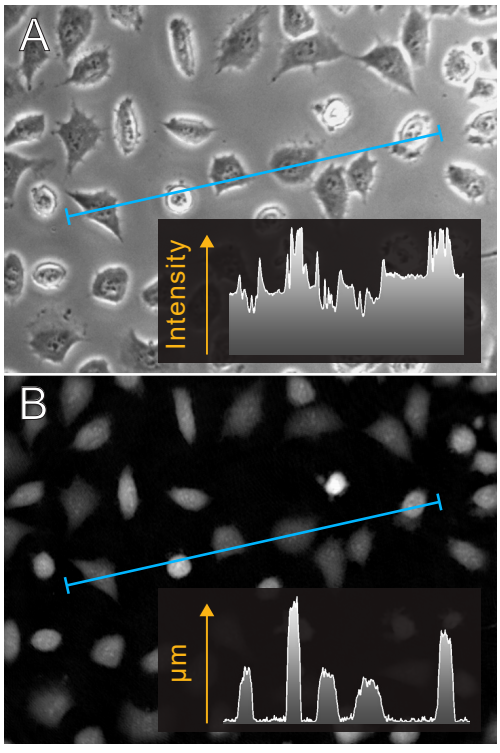
With computer technology, it is possible both to quantify and visualize phase-shifts. This technique is called *quantitative phase imaging* (QPI) or *quantitative phase contrast microscopy* to distinguish it from conventional phase contrast microscopy. QPI provides both quantitative and beautiful images of living cells, transforming phase microscopy and live cell imaging into a quantitative tool for detailed cell analysis.



An example of a quantitative phase image of cells in 3D, created by HoloMonitor. The height of the cell and its color tone correspond to the optical thickness of the cell.

QUANTITATIVE PHASE IMAGING VS. PHASE CONTRAST

To illustrate the difference between quantitative phase and phase contrast microscopy images, the same living cells were imaged with both modalities (right). As can be been seen from the intensity profiles, the individual cells are much more easily singled out from the less confusing background in the latter quantitative phase image (B).

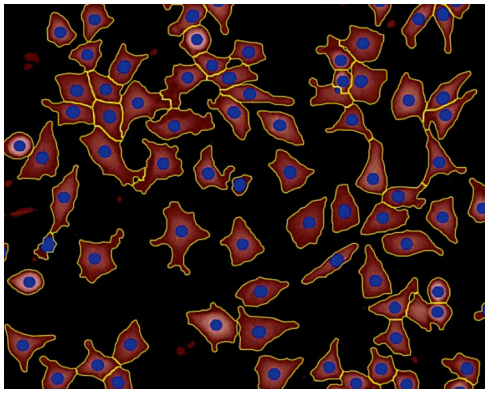


Humans are exceptionally good at processing visual information. The characteristic bright halo seen around cells in a phase contrast image does not bother us humans. However, computers process images very differently from us humans and rely on that the cells are distinctly separated from the background, as computers have no prior knowledge of what a cell looks like.

We have similar limitations when we are asked to find an object that we do not know how it looks like. Unless it is very obvious what distinguishes the object from the surrounding background, we will not be able to identify the object.

Simpler live cell identification

As the cells are well separated from the background in a quantitative phase image, simpler and more robust computer algorithms can be used to identify individual cells in such images.



Cells identified in a quantitative phase image recorded by HoloMonitor.

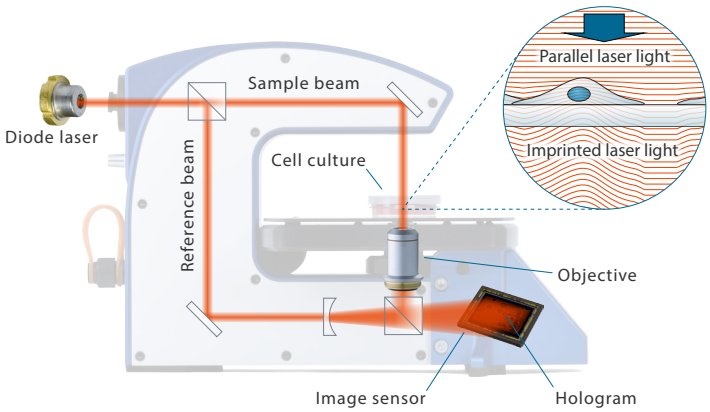
Optical thickness

Unlike in phase contrast microscopy images, the intensity of a pixel in a phase image has a direct physical meaning. It corresponds to the optical thickness of the cell, which is the physical height of the cell multiplied by the optical density of the cell at that point. Consequently, cell structures that are optically dense like lipid droplets will appear as bright spots. Reversely, less dense objects like vacuoles will appear as dark spots within the cell.

When light waves interact they create an interference pattern, just like water waves do.

HOLOGRAPHIC MICROSCOPY

There are several forms of quantitative phase microscopy. The HoloMonitor® live cell time-lapse cytometers[1] employ the most common form, *holographic microscopy.*



Shallow water imprinted beach waves

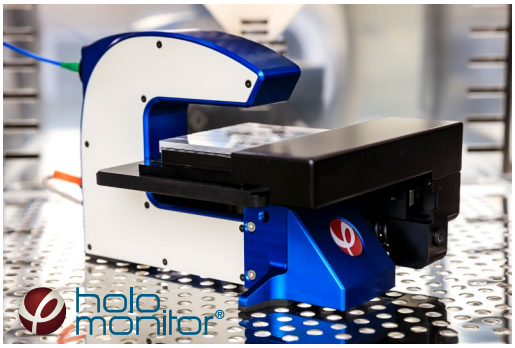
Holographic microscopy creates quantitative phase images by letting a sample beam and a reference beam interfere to create an interference pattern or *hologram,* as shown above. The hologram is recorded by an image sensor and computer processed to produce the phase image.

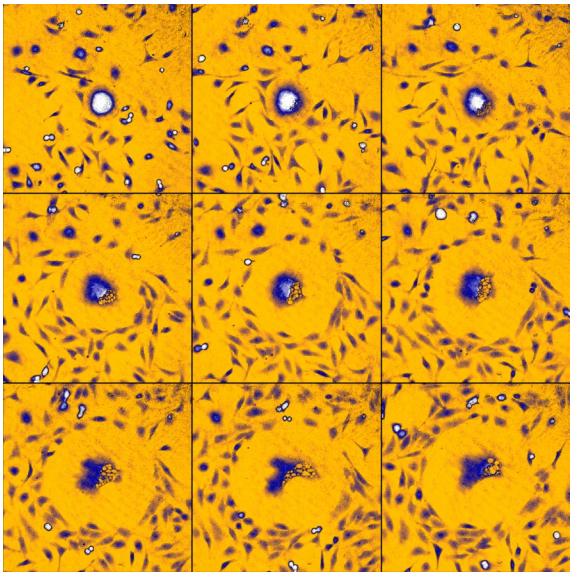
Is is useful to think of a phase image as a picture of the optical imprint created by the cells. When the illuminating sample beam passes through the sample, the sections of the beam that passes through the more optically dense cells are delayed in relation to the background. This shifts the phase of the parallel sample beam and imprints the morphology and 3-dimensional optical properties of the cells on the sample beam, similar to how beach waves are delayed and phase shifted when they reach shallow water.

TIME-LAPSE CYTOMETRY

Understanding cellular dynamics

Another advantage of holographic microscopy is that the created quantitative phase images are focused when viewed, not when recorded. This makes HoloMonitor ideal for long-term imaging and analysis of living cells by means of time-lapse microscopy, which acquires a series of cell images at regular time intervals to analyze the dynamics of various cellular events. Unfocused images, caused by focus drift, are simply refocused by letting the computer software recreate the phase image from the recorded hologram.





An example time-lapse image sequence of a monster HeLa cell, imaged by HoloMonitor. HeLa cells are cancer cells named after Henrietta Lacks, who in the early 1950s donated the first cells that was successfully kept alive and cultured in a laboratory environment. HeLa cells and other immortal cells are today routinely cultured by scientists to study the complex behavior of cells and their response to drug treatments.

In addition to identifying each individual cell, HoloMonitor provides data for analysis of more than 30 morphological parameters. However, the true power of time-lapse cytometry first emerges when the same cells are monitored over time. The HoloMonitor design utilizes recent technological advances to allow time-lapse image sequences of cultured cells to be effortlessly recorded over long time periods.

With HoloMonitor installed in a cell incubator, the cells are kept in a cell friendly environment during the entire experiment. Long-term live cell kinetic data can easily be obtained using time-lapse imaging. Images are recorded at selected intervals, down to 1 image/sec. Depending on the application, cell images are played back as a video recording to aid analysis of dynamic cell behavior.

HoloMonitor App Suite

From recorded time-lapse sequences, the HoloMonitor App Suite software helps the user to automatically extract and kinetically analyze live cell population data based on individual cell data. When preferred, individual cell data – such as cell count, cell morphology, cell velocity, and cell division rate – can all be measured from the same experimental time-lapse data, without requiring additional experiments.

When HoloMonitor was compared with standard transwell migration and invasion assays, the tracking of non-directional motility of individual cells over time produced highly correlated relative motilities.

Evaluation of holographic imaging cytometer HoloMonitor® M4 motility applications, Cytometry (2018)
— **Yuntian Zhang and Robert L. Judson**, University of California, San Francisco

LIVE CELL IMAGING & ANALYSIS ASSAYS

Altogether the described features enable a range of biologically relevant parameters to be effortlessly analyzed over time. The label-free live cell imaging and analysis technology, together with the incubator compatible design, ensures that the living cells are studied in a cell friendly environment avoiding artifacts and photo-toxicity. The following live cell assays are available:

Cell proliferation: Traditional methods are end-point assays that often assess cell proliferation indirectly, or only based on cell confluence measurements. HoloMonitor offers a convenient assay that automatically presents kinetic cell proliferation data. Cell proliferation is directly determined; both by cell counting and by confluence assessment.

Cell health/QC: HoloMonitor can easily be used to explore cell status and quality prior to an experiment to ensure the correct cell number is seeded, as well as to follow cell growth in terms of cell count and cell confluence, as the study progresses.

Dose response: Responses to various drugs and treatments are often studied using cell proliferation curves only. Using HoloMonitor it is also possible to observe and analyze events that precede deviant proliferation. The range of morphological parameters can often reveal details and early signs of cell death and adds information on how the cell dies.

Cell movement: The identification of single cells, enables detailed analysis of the motility and migration of cell populations. Since the cell population data is based on individual cell data, subpopulations of differently behaving cells can be identified and analyzed separately[3].

Moreover, since HoloMonitor leaves the cells unharmed additional studies on the cells, or the supernatant, can be performed after imaging. Visit www.phiab.com/applications to explore our full list of applications.

REFERENCES

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All HoloMonitor related publications are available here.