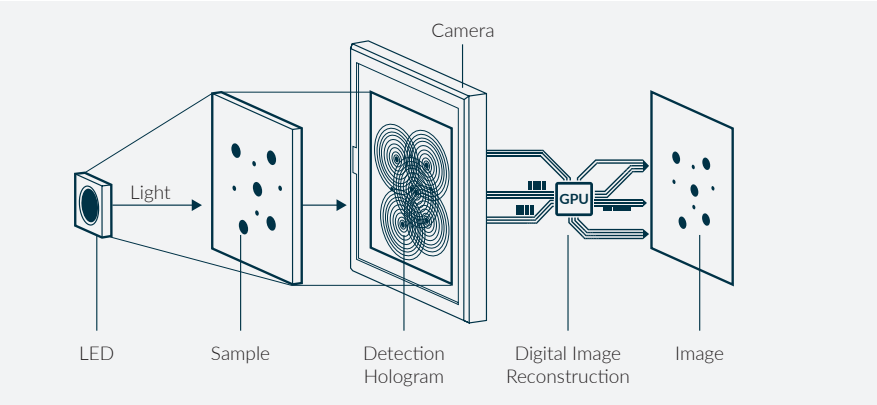
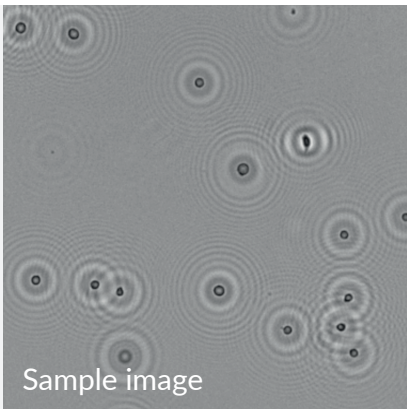
Staining-free cell viability analysis with the fluidlab R-300

The fluidlab R-300 uses the label-free technique of digital holographic microscopy (DHM) to analyze cell number and viability directly in the cell culture environment. Contrary to brightfield microscopy, DHM does not use optical lenses but generates so-called 'holograms' of the cells, which are analyzed by a computer. Importantly, these holographic images contain information on both outer cell morphology and cytoplasmic composition based on differences in refractive indices of cellular structures. This property makes the DHM imaging technology suitable for sensitive measurements of various cellular events such as cell death.

What is digital holographic microscopy?





Digital holographic microscopy (DHM) is a state-of-the-art quantitative phase imaging technique that allows automated label-free analysis of cell counts and viability. In DHM, the sample is illuminated with light. As light passes through the sample, some of it gets diffracted according to its refractive index while some travels through without 'seeing' the sample. After the sample, the diffracted light interacts with the non-diffracted light, thus creating a hologram as it hits the camera. The hologram is then reconstructed digitally to retrieve an image, which contains valuable information about the cell culture sample such as membrane integrity and protein content.

What is the difference to "standard" brightfield microscopy?

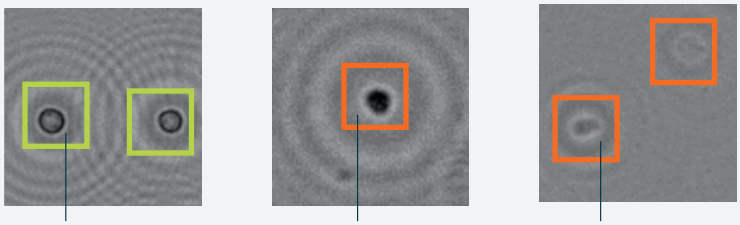
Brightfield microscopy uses lenses to create images based on the absorption of light by the specimen. Many biological samples are naturally colorless and poor in contrast as they absorb only little light. To increase contrast, researchers often use staining to visualize their sample with brightfield microscopy.

In DHM, that does not use lenses at all, images are formed based on the refractive index of the sample. The refractive index of a biological structure determines the way it interacts with light. It contains information about the difference of optical density at interfaces like different membranes or protein-rich compartments. That is the reason why, a holographic image has more information content then a brightfield image of comparable resolution. However, this information is not always easily interpretable by the human eye. Therefore, the fluidlab R-300 uses Convolutional Neural Networks (CNNs) to classify cellular states such as cell death

How can cell death be visualized with DHM?

Cell death processes are accompanied by dramatic changes in cell morphology, e.g. volume changes and fragmentation. In vitro assays typically distinguish between viable and non-viable cells by assessing membrane integrity through inclusion of dyes such as trypan blue. However, cell staining may induce cytotoxic effects following long exposures to the reagent leading to error-prone estimates of cell viability.

HeLa cells (size: approx. 40 µm):



Alive Cell
Intact membrane that clearly separates cyto-plasm from surrounding

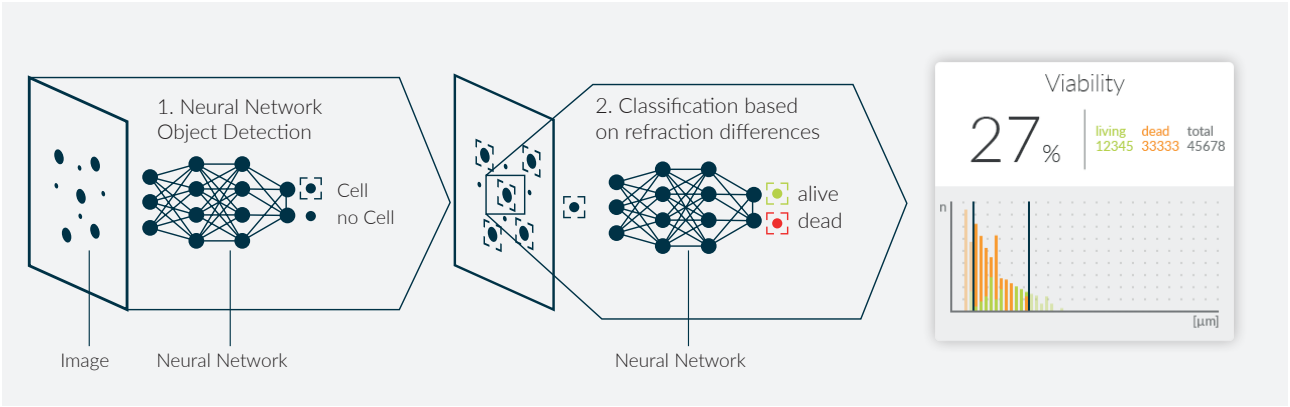
Early Cell Death
Losing of intracellular features

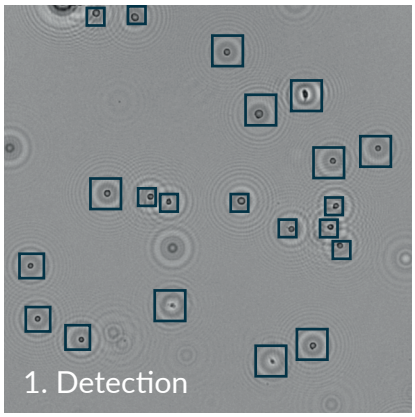
Late Cell Death
Losing of boundaries and intracellular protein content

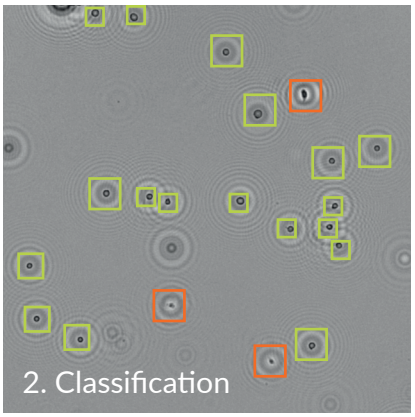
In contrast, the label-free DHM technique highlights the morphological and structural changes induced during cell death. Qualitatively, living cells are characterized by a dark contour resembling the intact cell membrane and a bright, structured cytoplasm. Dead cells, instead, initially appear as dark objects that later loose contrast and a well-defined boundary due to processes such as membrane fragmentation, karyolysis (fragmentation of the nucleus membrane) and leakage of the cytoplasm.

How does the fluidlab R-300 analyze cell viability?

The fluidlab R-300 uses supervised machine learning algorithms (the CNNs) to detect and count all cells within the image based on their morphology. Moreover, every cell is analyzed by a CNN to determine its state of cell viability. The determination of cell viability with DHM relies on a signal that is proportional to the intracellular refractive index as well as the membrane contrast. The fluidlab R-300 analyzes three classes for viability: alive, dead, and so called “non-interpretable” cells. Non-interpretable cells are cells that seem to behave differently or that could not be analyzed with high confidence by the CNN. This third cell class is not included in the total cell count nor in the determination of the viability ratio.







At the end of the analysis, the percentage of viable cells in the sample and the absolute numbers of dead and alive cells are displayed. Moreover, the boxed cells (colored-coded as live and dead) are displayed in the reconstructed holographic image. A histogram shows the number of alive and dead cells as a function of their size. It is possible to manually adjust the size range to include only cells of a certain size into the analysis. It is to be expected that the average size of the live and dead cell population can particularly vary, due to shrinking effects and other changes in morphology.

In summary, the fluidlab R-300 enables label-free measurements of cell viability just as efficiently and precisely as standard staining techniques.

- Small size – Viability analysis everywhere
- Simplicity - No staining required
- Small Sample volumes <= 20µL
- Automated analysis to increase statistical certainty
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