Cell death with Quantitative Phase Imaging

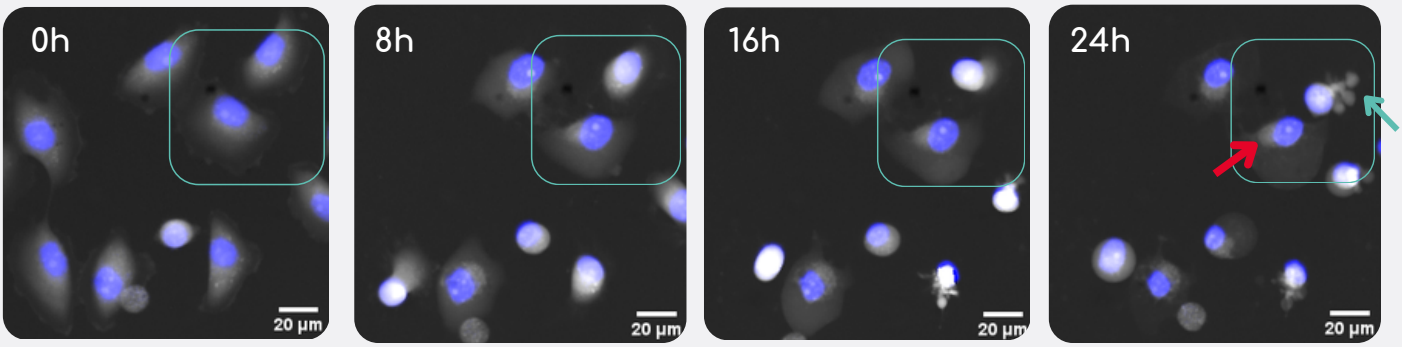
Cell death plays a crucial role in maintaining homeostasis within an organism by regulating the cell population. We distinguish different types of cell death based on morphological changes – **non-lytic** (apoptosis) and **lytic cell death** (e.g. necrosis). **Quantitative Phase Imaging (QPI) technology enables precise detection and quantification of changes associated with morphological and dynamic parameters.** This advanced technique allows for monitoring the behavior of single cells over time, observing cell reactions to drug treatments or stress conditions, and assessing the fraction of dead and viable cells within a large cell population based on measuring cell mass changes.

See cells undergoing apoptosis and necrosis in real time

Apoptosis is marked by distinct morphological changes, including cell shrinkage, where the cell reduces its own area and condenses chromatin. This is often followed by membrane blebbing (the "dance of death") and apoptotic body formation. Necrosis is characterized by cell swelling, a gradual increase of the cell area, and followed by the rupture of the plasma membrane [1]. With Q-Phase these changes can be observed in real time.

Combining QPI with fluorescence imaging for cell death detection

In addition, Q-Phase is a comprehensive tool that **integrates QPI with standard fluorescence-based assay**. Combining innovative label-free detection with proven fluorescent live/dead cell viability assays allows the study of time-dependent morphological changes and the simultaneous confirmation of specific processes. Fluorescence signals provided by different dyes serve as specific markers for detecting key cellular events, such as identifying the activity of executioner caspases or tracking nuclear changes [2]. By combining label-free QPI with fluorescence markers, researchers can achieve more precise detection and validation of results.



An example of fluorescence images merged with QPI of LNCaP cells treated with doxorubicin (DOX), captured over 24 hours. DAPI was used for staining cell nuclei. The turquoise arrow shows a typical apoptotic cell with apoptotic bodies and the red arrow points to a necrotic cell with a ruptured cell membrane. 10x magnification.

Apoptosis example video

telight

References

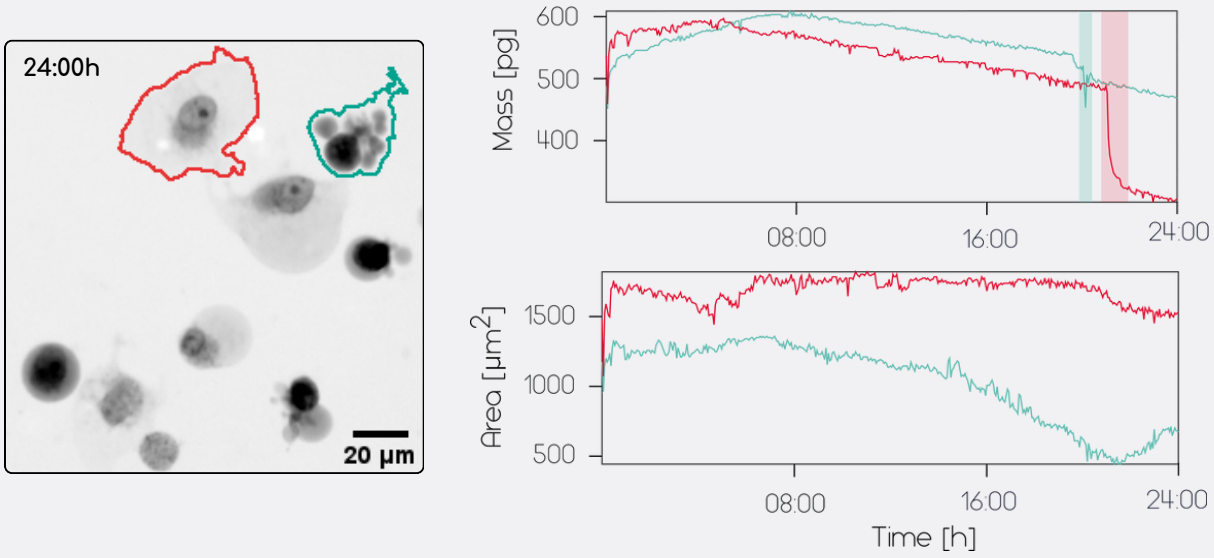
[1] Balvan, J. et al. Multimodal Holographic Microscopy: Distinction between Apoptosis and Oncosis. PLOS ONE 10, e0121674 (2015).

[2] Vicar, T., Raudenska, M., Gumulec, J. & Balvan, J. The Quantitative-Phase Dynamics of Apoptosis and Lytic Cell Death. Sci. Rep. 10, 1566 (2020).

Morphological and dynamic parameters for apoptotic and lytic cell death distinction

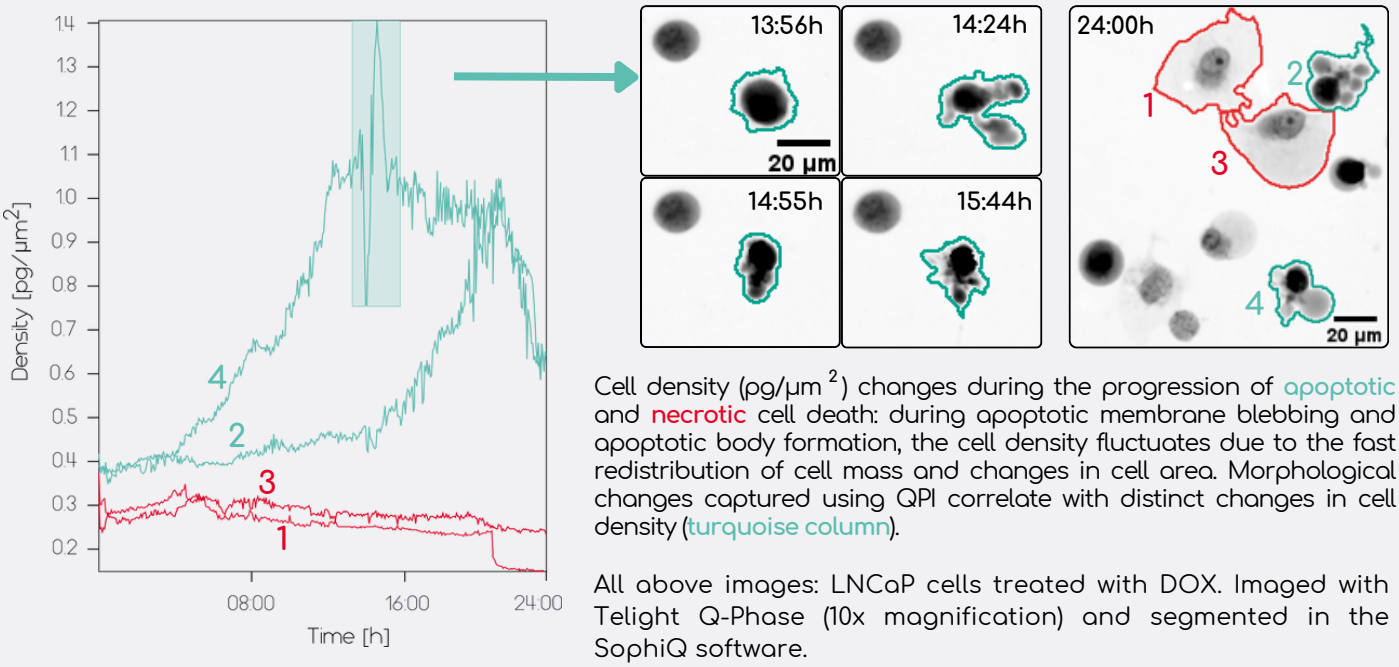
QPI enables the long-term observation of subtle changes in the cell mass distribution that are unrecognizable to the naked eye. Thanks to our technology, which utilizes an incoherent light source (LED), the images captured by Q-Phase give a clear background without parasitic interference or halo effects which allows for better cell segmentation crucial for further analysis. Additionally, the advanced segmentation features of the software SophiQ enable the tracking of cells over time and the calculation of various morphological parameters such as cell mass, density, area, circularity, Euclidean length, etc.

Changes in cell mass and area during apoptotic and necrotic cell death



During apoptosis, the **mass** increases due to higher metabolic activity, whereas the **area** decreases due to cell shrinkage caused by the loss of water and the condensation of cytoplasmic and nuclear components like chromatin. Then, apoptotic blebbing (marked by the turquoise column) and the formation of apoptotic bodies follow. In contrast, during necrosis, the cell dry **mass** declines and its **area** slightly increases due to initial cell swelling. Later, the cell membrane loses its integrity and the whole process is terminated by membrane rupture and a sheer drop in cell mass (red column).

Changes in cell density during apoptotic and necrotic cell death



Cell density (pg/µm ^{2}) changes during the progression of apoptotic and necrotic cell death: during apoptotic membrane blebbing and apoptotic body formation, the cell density fluctuates due to the fast redistribution of cell mass and changes in cell area. Morphological changes captured using QPI correlate with distinct changes in cell density (turquoise column).

All above images: LNCaP cells treated with DOX. Imaged with Telight Q-Phase (10x magnification) and segmented in the SophiQ software.

telight