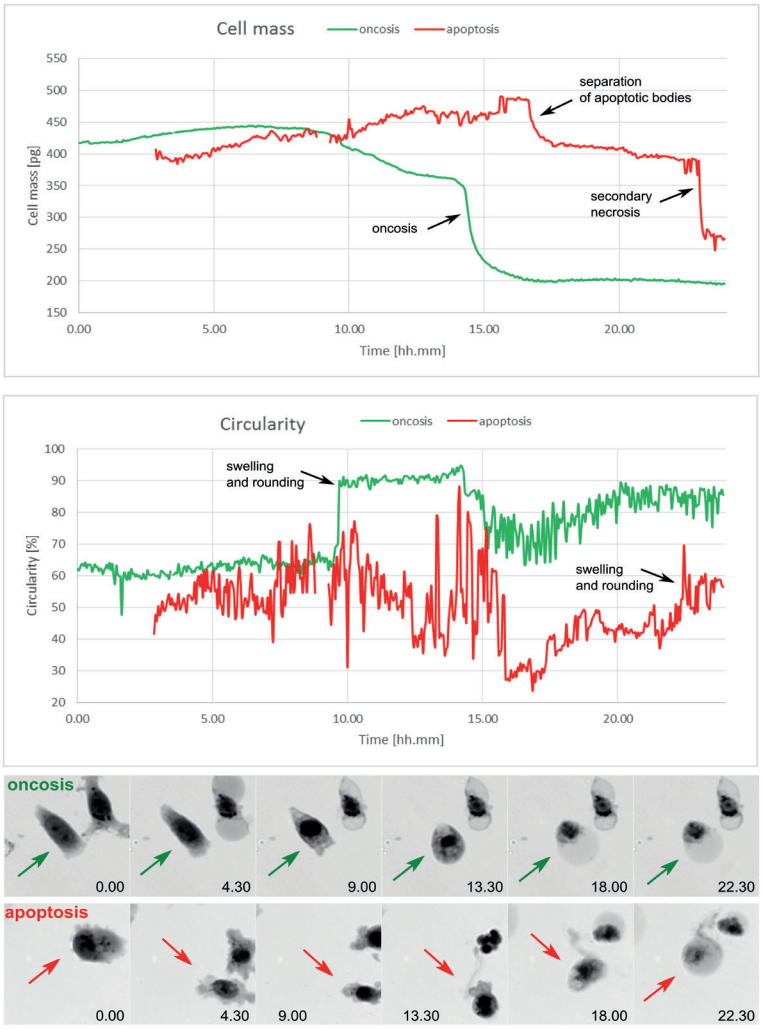
Application Example

Q-PHASE
CANCER RESEARCH

Quantitative Phase Imaging (QPI) provided by Q-PHASE – TESCAN multimodal holographic microscope – allows observation of cells reactions to different treatments without any other added dyes. Combination of QPI with fluorescence provides the possibility to observe cell processes with low phototoxicity and simultaneously verify observed processes using a single instrument. This innovative approach opens interesting opportunities in cancer research.

Through experiments, researchers look at how cancer cells behave and try to understand cancer at its deepest levels. Understanding the basic processes of these cells can help researchers figure out what controls cell division and cell death; find out what makes cancer cells spread or metastasize, identify unique characteristics of cancer cells to design new therapeutic strategies, and, find out why certain cancer cells become resistant to therapy. In some of these experiments, cells taken from tumors of people with cancer are studied.



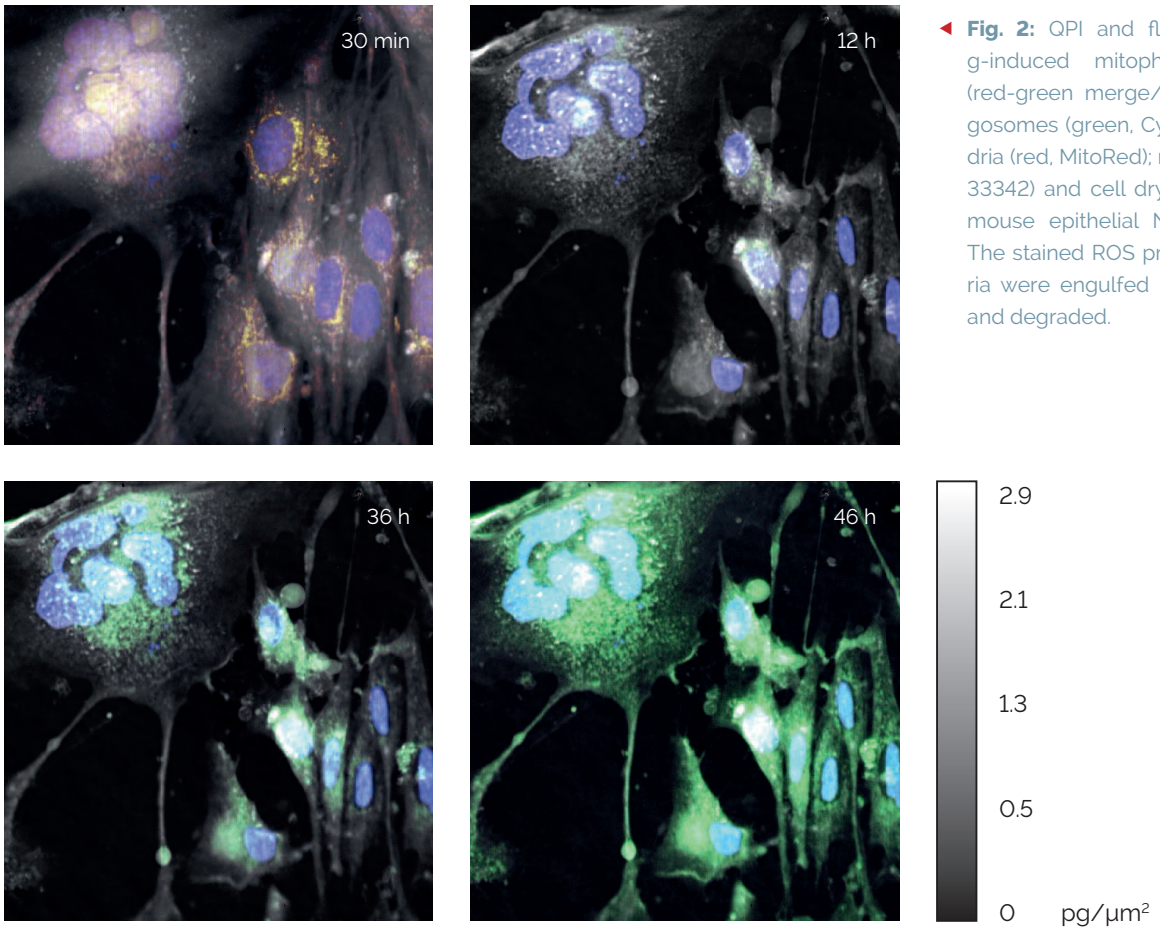
Cell Death Type Detection

Identification of the exact type of cell death following the cell injury is important for diagnostics, dose-response, and toxicological studies.
Using Q-PHASE, distinction between apoptosis and oncosis according to changes in morphological parameters is possible.

◀ **Fig. 1:** QPI evaluation of morphological parameters for oncosis and apoptosis. Oncosis (green) is characterized by cell swelling and following rupture of cell membrane. Apoptosis shows cell shrinking and formation of apoptotic bodies. In the absence of immune cells, apoptotic cells finally undergo secondary necrosis (decrease of cell mass and increase of circularity) [1].

How Do Cancers Become Chemoresistant?

Resistant cancer phenotype is a key obstacle in successful therapy of prostate cancer. We found out that autophagy (namely mitophagy) is an important resistance mechanism. The major reactive oxygen species (ROS) producing mitochondria were coated by an autophagic membrane derived from endoplasmic reticulum and degraded [2]. Most of fluorescent dyes cause generation of ROS too. Phototoxicity in fluorescence live-cell imaging is mainly induced by excited fluorophores, which produce ROS. ROS react with a large variety of easily oxidizable components, such as mitochondrial proteins leading to loss of fluorescence signal (photobleaching) and cell cycle arrest or cell death (phototoxicity).



◀ **Fig. 2:** QPI and fluorescence labeling-induced mitophagy. Colocalization (red-green merge/yellow) of autophagosomes (green, CytoID) and mitochondria (red, MitoRed); nuclei (blue, Hoechst 33342) and cell dry mass (grey, QPI) in mouse epithelial NMuMG Fucci cells. The stained ROS producing mitochondria were engulfed by autophagosomes and degraded.

Identifying unique characteristics of cancer cells.

For decades, authors have described unusual cell structures, referred to as cell-in-cell structures, in which whole cells are found in the cytoplasm of other cells. One well-characterized process that results in the transient appearance of such structures is the engulfment of apoptotic cells by phagocytosis. However, many other types of cell-in-cell structure have been described that involve viable non-apoptotic cells. Some of these structures seem to form by the invasion of one cancer cell into another, rather than by engulfment [3].

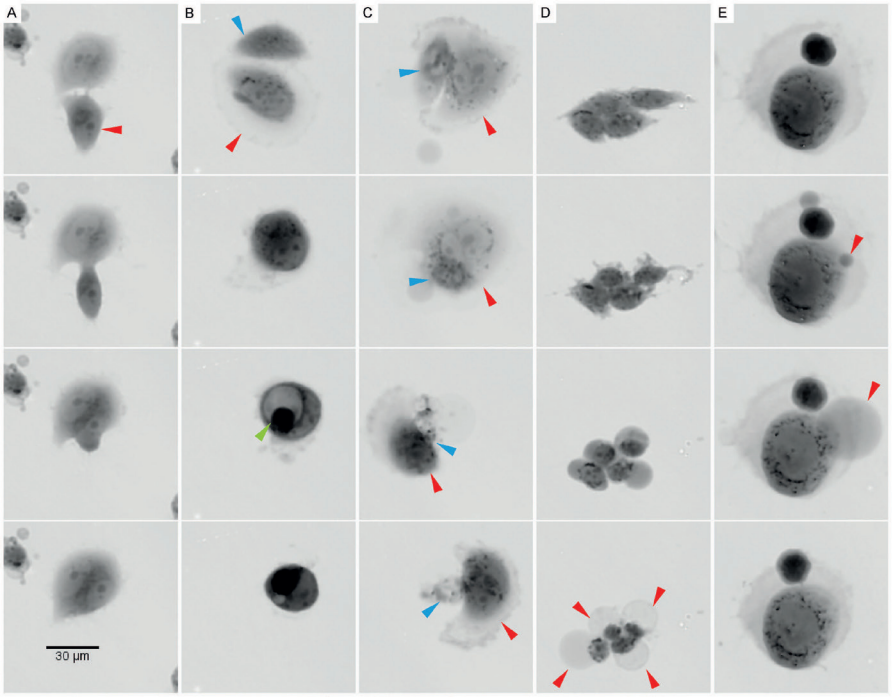


Fig. 3: QPI time-lapse of cell interactions. **(A)** Time-lapse imaging of entosis; internalized cell (red arrow) played an active role in its engulfment, which resulted in complete internalization. **(B)** Time-lapse imaging of cell fusion with cannibalism (digestion of engulfed cell); during fusion-cannibalism of living cells, the cannibal cell (red arrow) came in contact with the target cell (blue arrow). A bird-eye structure was observed as a consequence of target cell vacuolization (see green arrow). **(C)** Time-lapse imaging of cannibalism without fusion; the dying cell (blue arrow) was attacked and exploited by the cannibal cell (red arrow). The target cell was dead after the attack. **(D)** Time-lapse imaging of oncosis; oncotic cells formed typical cytoplasmic blebs that usually lead to necrosis (see red arrow). **(E)** Time-lapse imaging of reverse oncosis; initial forming of oncotic blebs (see red arrow) did not lead to necrosis; the bleb was absorbed and the cell remained viable.

Conclusion

Q-PHASE allows capturing cell population behaviour in real-time. In combination with classical molecular and biochemical analyses, Q-PHASE provides a comprehensive view of cancer cell behaviour. The generated easily quantifiable data are a great source for publication, web presentations, and teaching.

References

1. Balvan J, Krizova A, Gumulec J, Raudenska M, Sladek Z, Sedlackova M, et al. (2015) Multimodal Holographic Microscopy: Distinction between Apoptosis and Oncosis. PLoS ONE 10(3): e0121674. doi:10.1371/journal.pone.0121674

2. Balvan J, Gumulec J, Raudenska M, Krizova A, Stepka P, Babula P, et al. (2015) Oxidative Stress Resistance in Metastatic Prostate Cancer: Renewal by Self-Eating. PLoS ONE 10(12): e0145016. doi:10.1371/journal.pone.0145016

3. Overholtzer M, Brugge JS. The cell biology of cell-in-cell structures. Nature Reviews Molecular Cell Biology 2008; 9:796-809.

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