Application Example

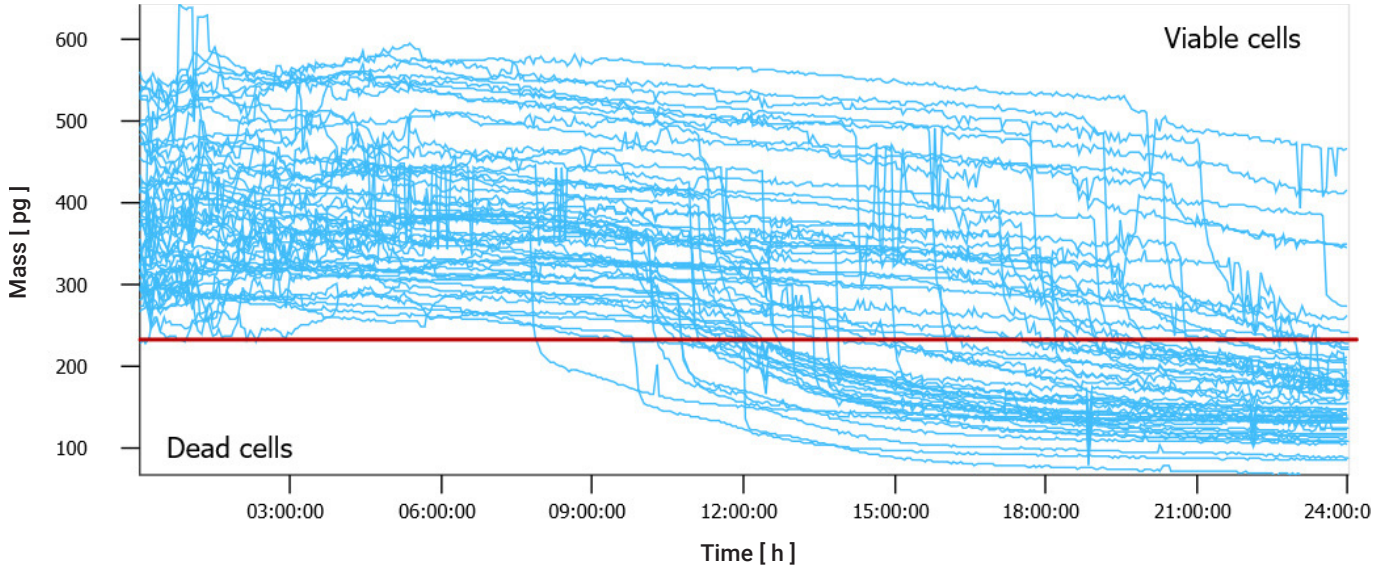
QPI

Q-PHASE
Non-invasive and label-free detection of cell death

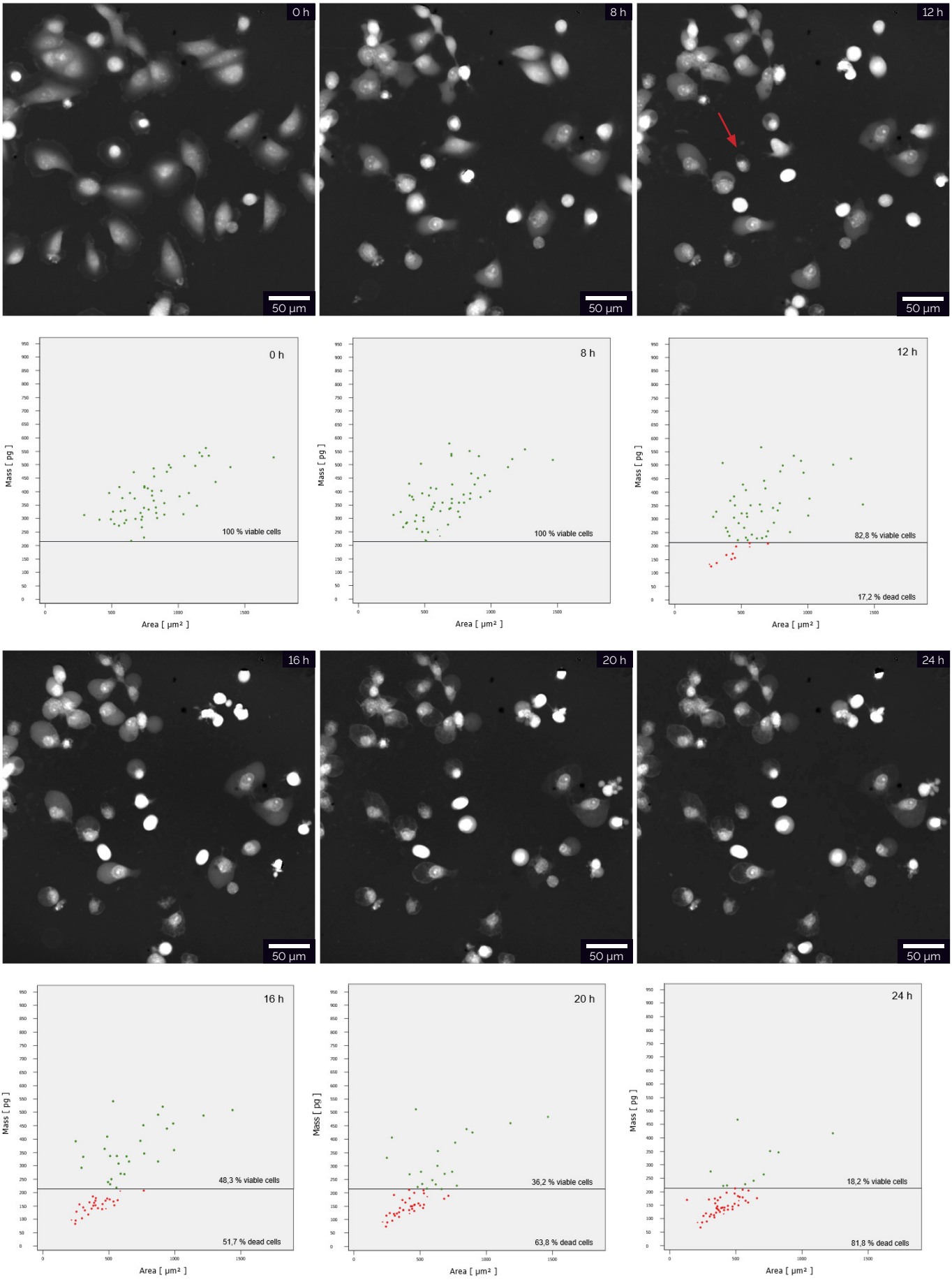
Quantitative phase imaging (QPI) provided by Q-PHASE, a TESCAN multimodal holographic microscope, allows precise detection and quantification of changes in cell dry mass and related morphological parameters specific for cell death [1, 2]. Thus, individual phases of cell death can be easily distinguished based on the monitoring of a single cell behavior over time. Using the Q-PHASE, significant changes of cellular parameters have been detected in human prostate adenocarcinoma LNCaP cells treated with a chemotherapy drug doxorubicin (DOX) [3].

Quantification of cell dry mass during cell death

Thanks to the unique segmentation capability of Q-PHASE analysis toolbox, a dry mass of a single cell can be measured with high precision. LNCaP cells were exposed for 24 hours to DOX (0.75 μM) and two major intervals were identified from the time changes of a cell dry mass (Fig. 1). After approximately 12 and 18 hours, a steep decline in mass was clearly visible for treated cells. Since this decrease is characteristic of cell death [4], it is possible to observe cell reactions to drug treatment or other stress conditions, and assess the fraction of dead and viable cells in a given cell population. For detailed depiction of morphological changes during the treatment of LNCaP cells with DOX, QPI images along with scatter plots for separate time points are shown in the Fig. 2.



▲ **Fig. 1: Time-lapse detection of cell dry mass changes in LNCaP cells treated with DOX.** The mass of the cells was significantly reduced as consequence of cell death processes. Based on the evaluation of morphological changes in whole cell population (see Fig. 2), a minimum mass limit for viable cells was set to 220 pg.



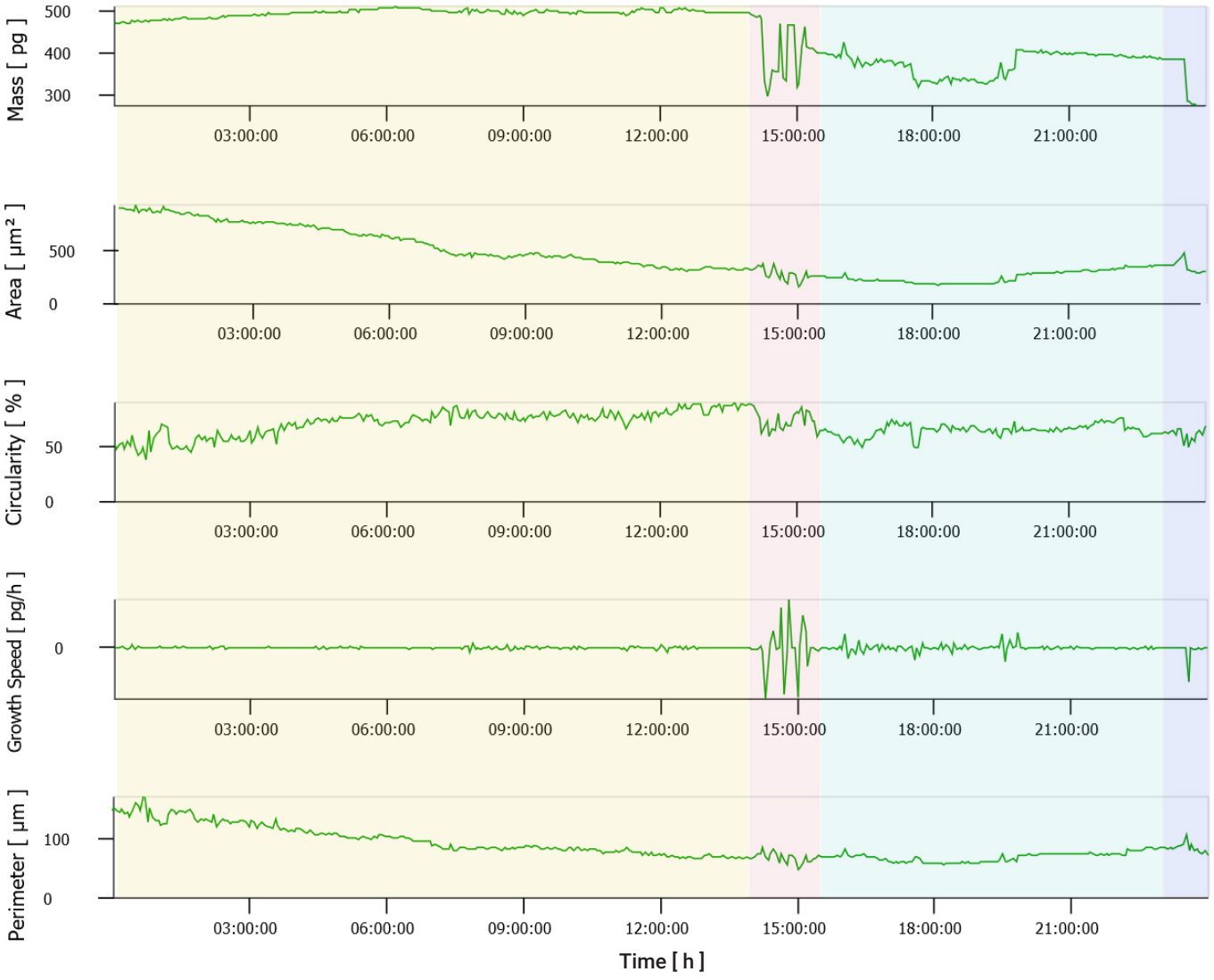
▲ **Fig. 2: Changes in morphological parameters of LNCaP cells treated with DOX.** Cells with small area and dry mass below the determined mass limit are considered dead (red dots). Red arrow indicates an example of dead cell in QPI image.

Distinction between apoptosis and necrosis

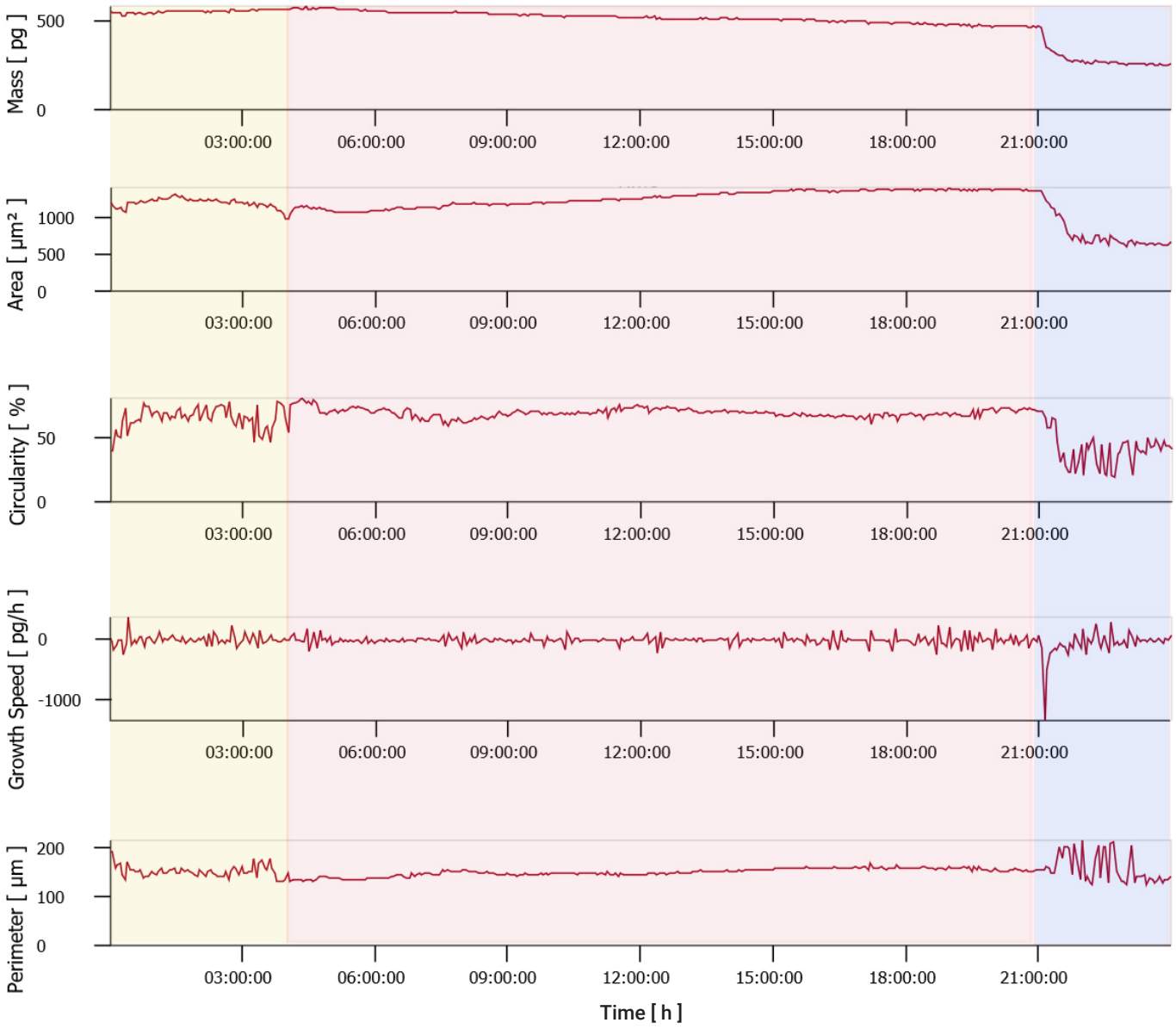
Since morphological changes caused by biochemical processes during apoptotic cell death are different from those caused by necrotic processes, it is possible to distinguish between these diverse types of cell death by monitoring morphological parameters of individual cells [5].

Apoptosis driven by a caspase activation is often accompanied by fluctuations in cell morphology and dynamics caused by the membrane blebbing and apoptotic body formation. With higher metabolic activity of cell prior to apoptosis, a cell dry mass slightly increases while its area decreases due to the cell shrinkage. Caspase-mediated cleavage of cytoskeletal actin causes cell rounding and increases circularity of the apoptotic cell (Fig. 3, yellow area). Aggregation and separation of apoptotic bodies creates a characteristic saw-toothlike pattern on corresponding time curves (Fig. 3, red area). When there is no intervention of scavengers, and the full apoptotic program is completed, an autolytic process of secondary necrosis with cell disintegration and steep decline of cell dry mass occurs (Fig. 3, green and blue area).

In the case of caspase-independent necrosis, cytoskeletal proteins remain intact, leading to different course of cellular parameters. After slight increase of mass caused by the cell metabolic activity (Fig. 4, yellow area), a cell loses control over itself which results in cell swelling and gradual increasing of cell area (Fig. 4, red area). Cell dry mass declines and whole process is terminated by a loss of cell membrane integrity with sheer drop of mass and negative growth speed (Fig. 4, blue area). Rapid changes in cell perimeter and circularity are result of an intracytoplasmic water accumulation and cell blebbing.



▲ **Fig. 3: Changes in morphological and dynamic parameters during apoptosis of LNCaP cell.** Yellow area denotes an interval of metabolic activity prior to apoptotic blebbing shown in red area. Green area denotes an interval of cell disintegration prior to secondary necrosis. Blue area indicates the secondary necrosis - a mode of cell elimination under impaired cell scavenging.



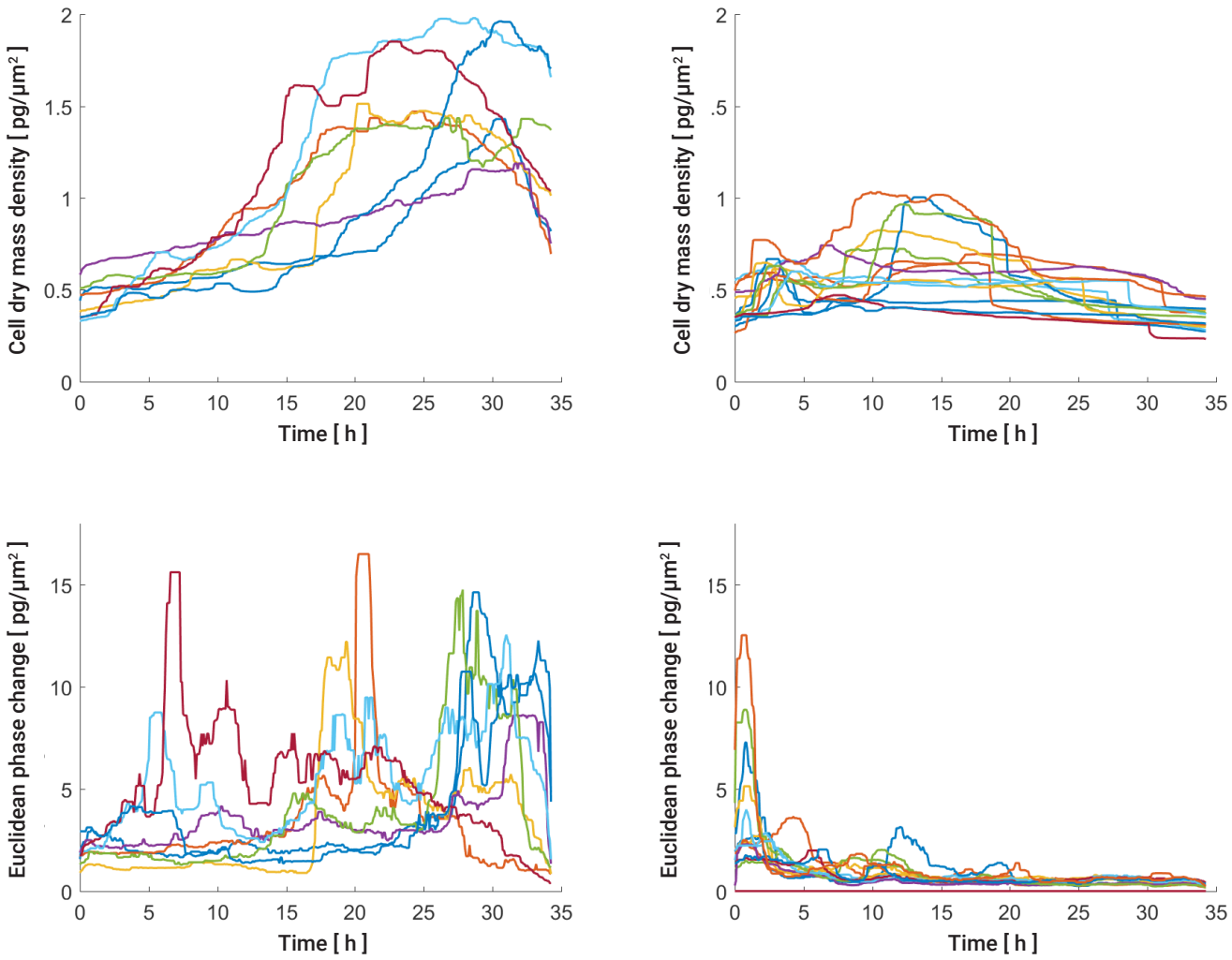
▲ **Fig. 4: Changes in morphological and dynamic parameters during necrosis of LNCaP cell.** Yellow area denotes an interval of metabolic activity before the cell loses control over itself. Red area indicates cell swelling. Blue area denotes a loss of cell membrane integrity with sheer drop of mass and negative growth speed.

Automatic classification of cell death

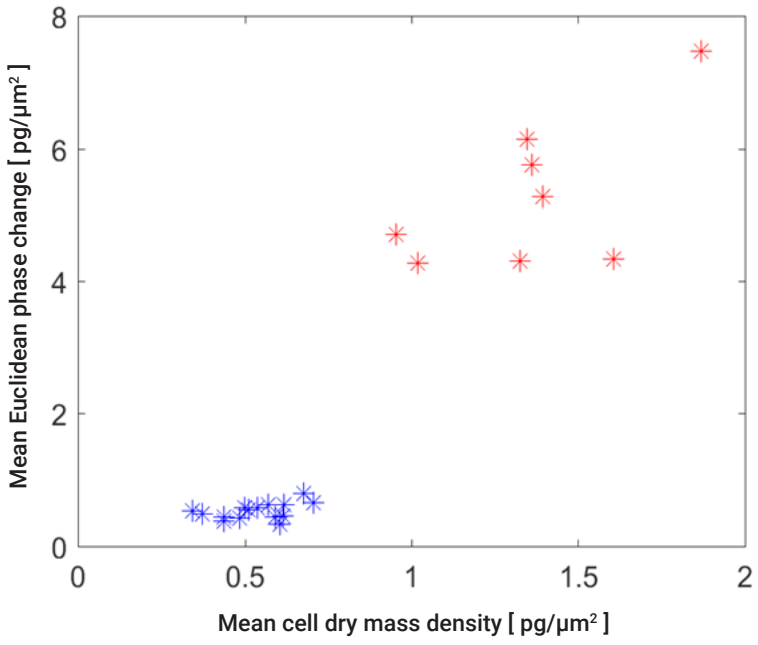
Images acquired by the Q-PHASE enable calculation of parameters that may vary significantly between different dying cells. Such parameters could allow for an automatic classification of cell death type. An example is a time course of changes in cell dry mass density, or Euclidean phase change of corresponding pixels calculated using the Euclidean distance metric for computing similarity between two successive images:

$$C_E(a,b) = |a-b| = \sqrt{\sum_{i=1}^{N}(a_i - b_i)^2}$$

Where *a* and *b* are phase values of corresponding pixels in two following frames. For apoptotic cells, both parameters extensively fluctuate, whereas remain relatively stable for cells that underwent necrosis (Fig. 5). A relationship between mean values of these two parameters for defined time interval affords an opportunity to distribute cell population automatically and assign a cell death type to individual cells (Fig. 6).



▲ **Fig. 5: Cell dry mass density and Euclidean phase change curves used for the automatic classification of cell death in population of LNCaP cells.** Left panel – apoptotic cells. Right panel - necrotic cells.



◄ **Fig. 6: Automatic classification of cell death based on the relationship between cell dry mass density and Euclidean phase change.** LNCaP cells were distributed into the group of necrotic (blue) and apoptotic (red) cells.

Conclusion

TESCAN Q-PHASE enables easy and fast detection of specific cell death without any need for labelling nor additional damage to cells. Thanks to the quantitative phase imaging and advanced analysis software, Q-PHASE provides precise information on morphological changes and dynamics of individual cells during cell death processes. Data obtained by Q-PHASE could help researches not only to distinguish between different types of cell death, but also to assess the rate of cell reactions to drug treatment or other stress conditions, and answer the questions of cancer cell behavior.

References

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