Application Example

Q-PHASE
Live Cell Imaging

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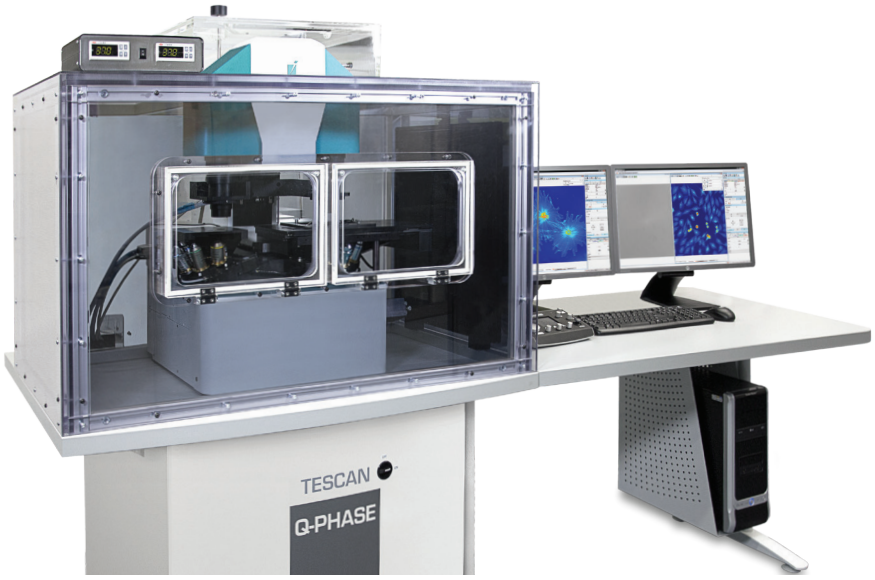
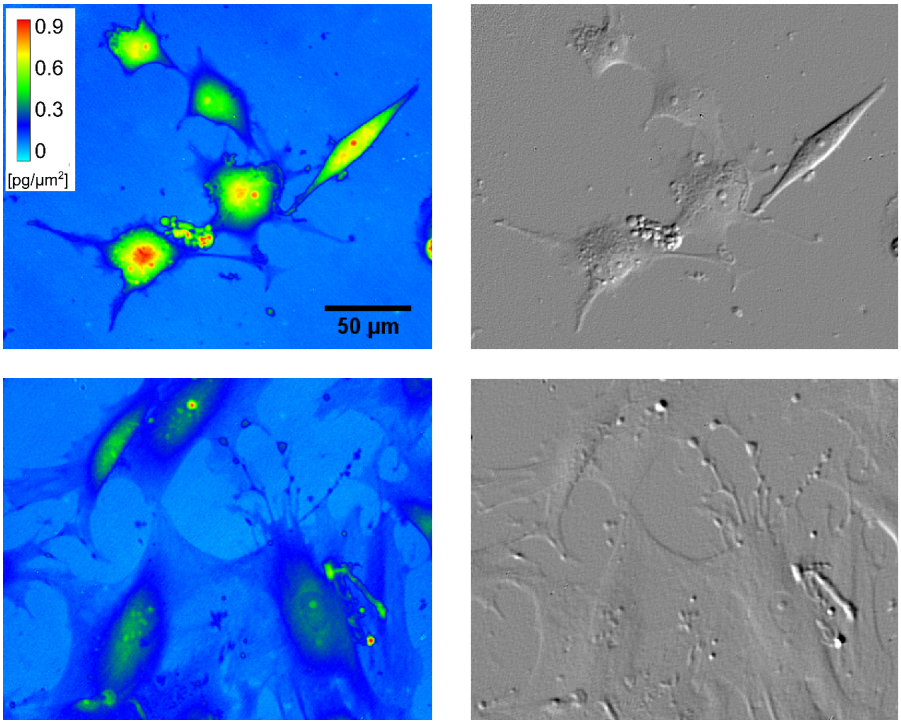
Q-PHASE, the multimodal holographic microscope, is a unique instrument for quantitative phase imaging (QPI) [1]. The main application of this technique is in live cell imaging where advantages such as no need for labeling, low phototoxicity, easy segmentation, cell dry mass interpretation of measured signal and suitability for long term experiments are used. Q-PHASE is built as a transmitted light microscope in an inverted configuration for easy handling with biological samples. Appropriate conditions for live cells are ensured by the microscope incubator heated to 37°C and low exposures of light for QPI. Moreover, there is no need for specific sample preparation. The cells are just seeded into a suitable observation chamber and examined.

Quantitative Phase Imaging (QPI)

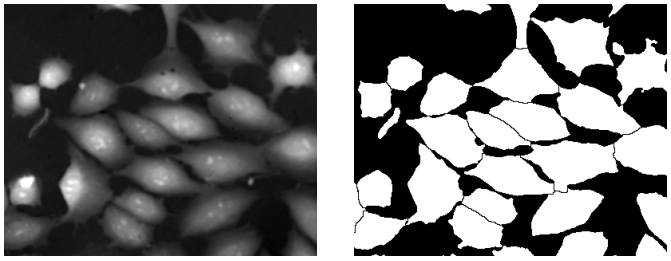
QPI is a new microscopy technique especially suited for live cell imaging, i.e. monitoring of cell reactions to treatment, analyses of movement, growth or various other characteristics. QPI provides images with high contrast without any staining. Information from QPI can be recalculated to cell dry mass [2, 3, 4]. The cell dry mass is a non-aquaeous material of a cell which mainly depends on the protein concentration and it has been defined as the weight of the cell when water has evaporated. The recalculation of the QPI signal to the cell dry mass is straightforward, the more cell dry mass is in the path of the light, the stronger the QPI signal is. Images acquired by Q-PHASE are free of artefacts such as halo, parasitic interferences or speckles which are usual in competing QPI instruments.



▲ **Fig. 1:** Q-PHASE, a multimodal holographic microscope with microscope incubator.



▶ **Fig. 2:** Comparison of quantitative phase images (left) and simulated DIC images (right). The imaged cells correspond to different lines of rat fibroblasts; line LW13K2 in the first row and line WAF in the second row.



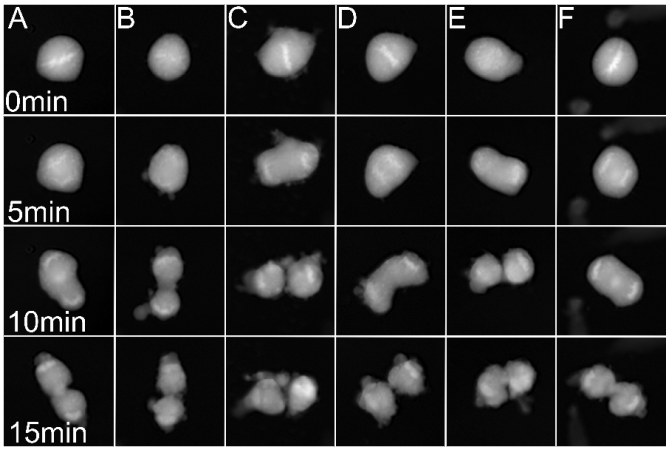
▲ **Fig. 3:** Segmentation of rat fibroblasts LW13K2 by the watershed method using the Q-PHASE software.

Segmentation

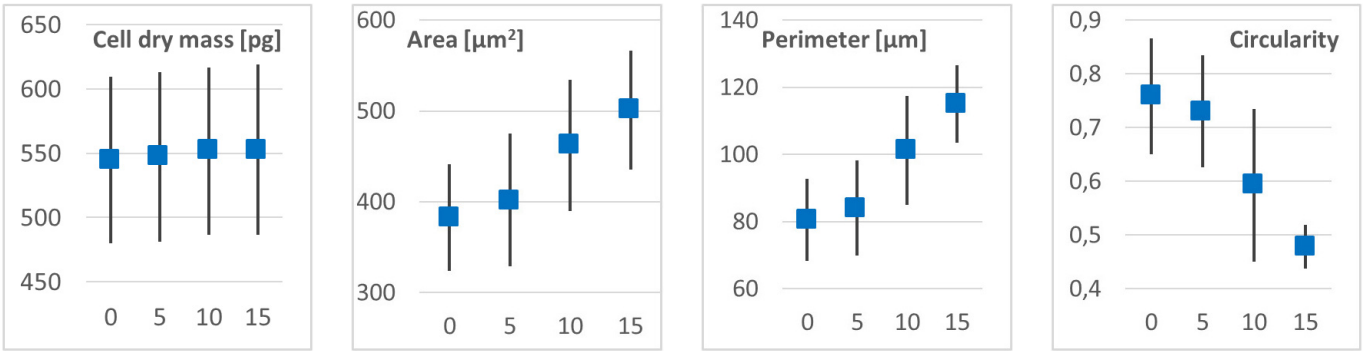
Owing to the high intrinsic contrast of QPI, cells are well distinguishable from the background, making the detection of cell boundaries and their segmentation easy. Segmentation of the cells from the background can be done by using simple thresholding or the watershed method which is especially useful when the cells are in contact. An illustration of segmentation on nearly confluent cell population is shown in Fig. 3. The segmented images enable the evaluation of various parameters.

Analysis

The set of mitosis observations presented in Fig. 4 was the basis for the evaluation of various parameters in four time points (Fig. 5).
The state with condensed chromosomes aligned to the metaphase plate was chosen as a reference time (0 min). At this point, the area and perimeter of the cells have low values and the shape of the cells is nearly circular. Later the chromosomes are split and pulled towards the opposite ends of the cells. The values of cell area and perimeter raise and the parameter of circularity decreases because of cell elongation. After that, both sets of chromosomes are surrounded by new nuclear envelopes and cytokinesis begins, identified by the cleavage furrow. During this process, major changes are identifiable in the area, perimeter and circularity parameters. The cell dry mass remains nearly constant over the whole process.



▲ **Fig. 4:** Selected time points (0, 5, 10, 15min) from the set of mitosis (A-F) of rat fibroblasts LW13K2.



▲ **Fig. 5:** Graphs of parameters evaluated in the selected time points during mitosis. Blue squares are mean values of observed cells. Black lines represent standard deviations.

Q-PHASE is a valuable tool for live cell imaging and analysis with its software enabling automated evaluation of a wide range of data in amounts sufficient for obtaining statistically significant results.

References

[1] Slabý T. et al.: Off-axis setup taking full advantage of incoherent illumination in coherence-controlled holographic microscope. Optics Express 21, 2013, 14747.
[2] R. Barer: Interference microscopy and mass determination. Nature 169, 1952, 366-367.
[3] H. Davies, M. Wilkins: Interference microscopy and mass determination. Nature 169, 1952, 541.
[4] R. Wayne: Light and video microscopy. Elsevier Science 2013.

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