Live cell microscopy with Quantitative Phase Imaging

Application note

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 research where advantages such as no need for labeling, low phototoxicity, easy segmentation, cell dry mass quantification 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.

QPI as an emerging technology

QPI is a novel microscopy technique especially suited for live-cell imaging, i.e. monitoring of cell reactions to treatment, analyses of their movement, growth etc. QPI provides images with high contrast without any staining and follows current trends of gentle and label-free imaging.

Cell dry mass quantification

The primary output data of this technology are expressed in units of radians. This data can be easily recalculated to cell dry mass in units of pg/μm^2 [2, 3]. The cell dry mass is all non-water content of a cell and consists mainly of proteins and lipids. The recalculation of the QPI signal to the cell dry mass is straightforward and is performed in real-time.

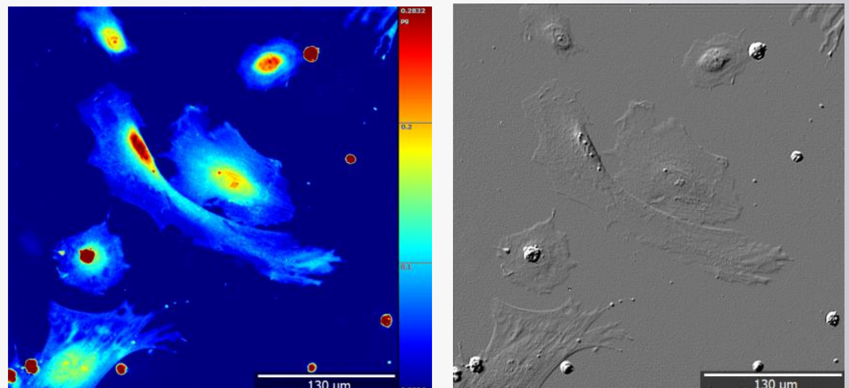
▲ Fig. 1: Q-Phase, multimodal holographic microscope for live cell QPI.

Image analysis without compromises

Due to an incoherent light source (LED), the images acquired by Q-Phase are free of artefacts (such as halo, parasitic interferences or speckles) commonly observed with other label-free imaging techniques. Moreover, these high-quality quantitative data are well suited for advanced image analysis using artificial intelligence.

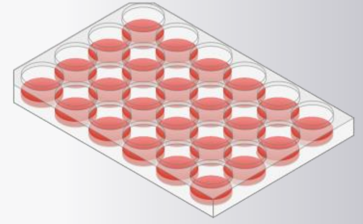
▶ Fig. 2: Comparison of quantitative phase images (left) and simulated DIC images (right).

Color-coded QPI shows the dry mass density in pg/μm^2 compared to the DIC image which is not quantitative. The imaged cells are mouse embryonic fibroblasts.

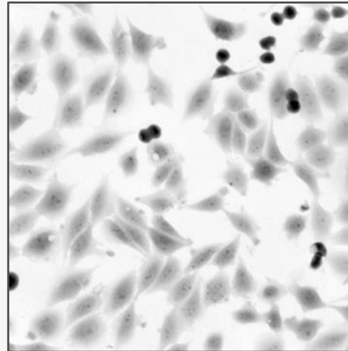


Experiment workflow

Observe Q-Phase is a fully automated live-cell imaging system equipped with an incubator for long-term experiments. The intuitive user interface allows to easily setup multiposition acquisition (including well plate formats), a combination of QPI and multiple fluorescence channels, with precise images ensured by an autofocus function.



Segment Owing to the high intrinsic contrast of QPI, cells are well discriminated from the background, making the detection of cell boundaries and their segmentation fast and easy.



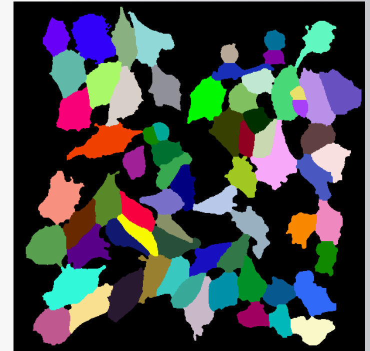


Fig. 3: Segmentation of rat fibroblasts LW13K2 using the Telight software.

Analyze Segmented data can be easily explored using the Telight software SophiQ. The set of mitosis is presented in Fig. 4 and quantitatively described in Fig. 5. Whereas the exemplary images show only selected timepoints (0 – 15 min), the corresponding time graphs describe the whole non-invasive monitoring of selected cells.

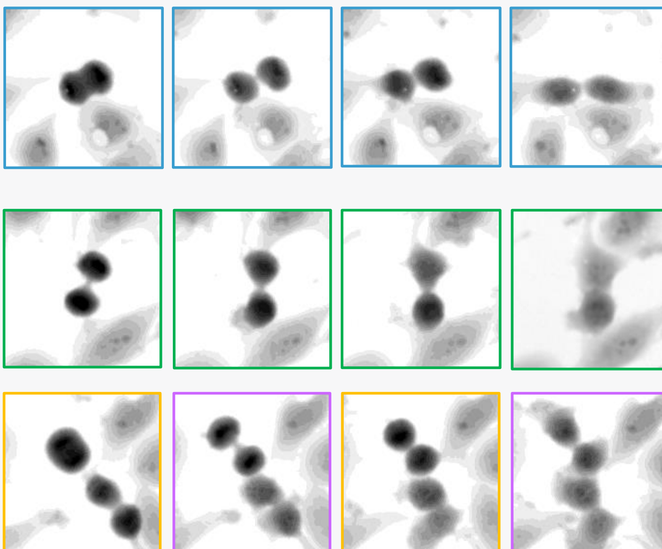


Fig. 4: Selected time points (5 min intervals) from the set of mitosis of rat fibroblasts LW13K2. Cytokinesis is followed by the cell elongation and cell density decrease.

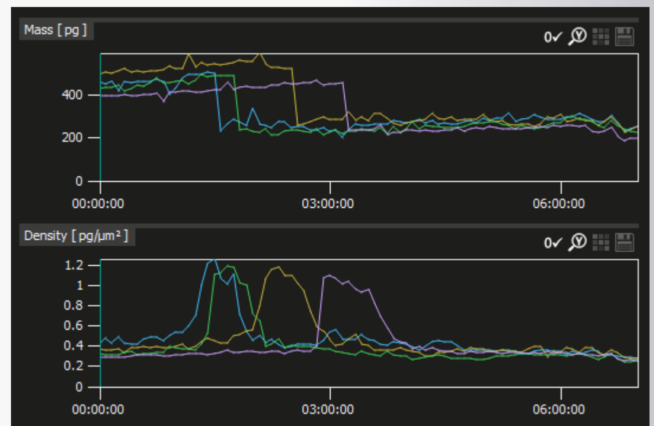


Fig. 5: Time graphs of cells undergoing mitosis detected in SophiQ software.

The upper graph shows the drop in cell dry mass after the cytokinesis; the lower graph shows the changes of cell density during mitosis. The highest cell density corresponds to the state of chromatin condensation.

Q-Phase microscope 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] R. Wayne: *Light and video microscopy.* Elsevier Science 2013.