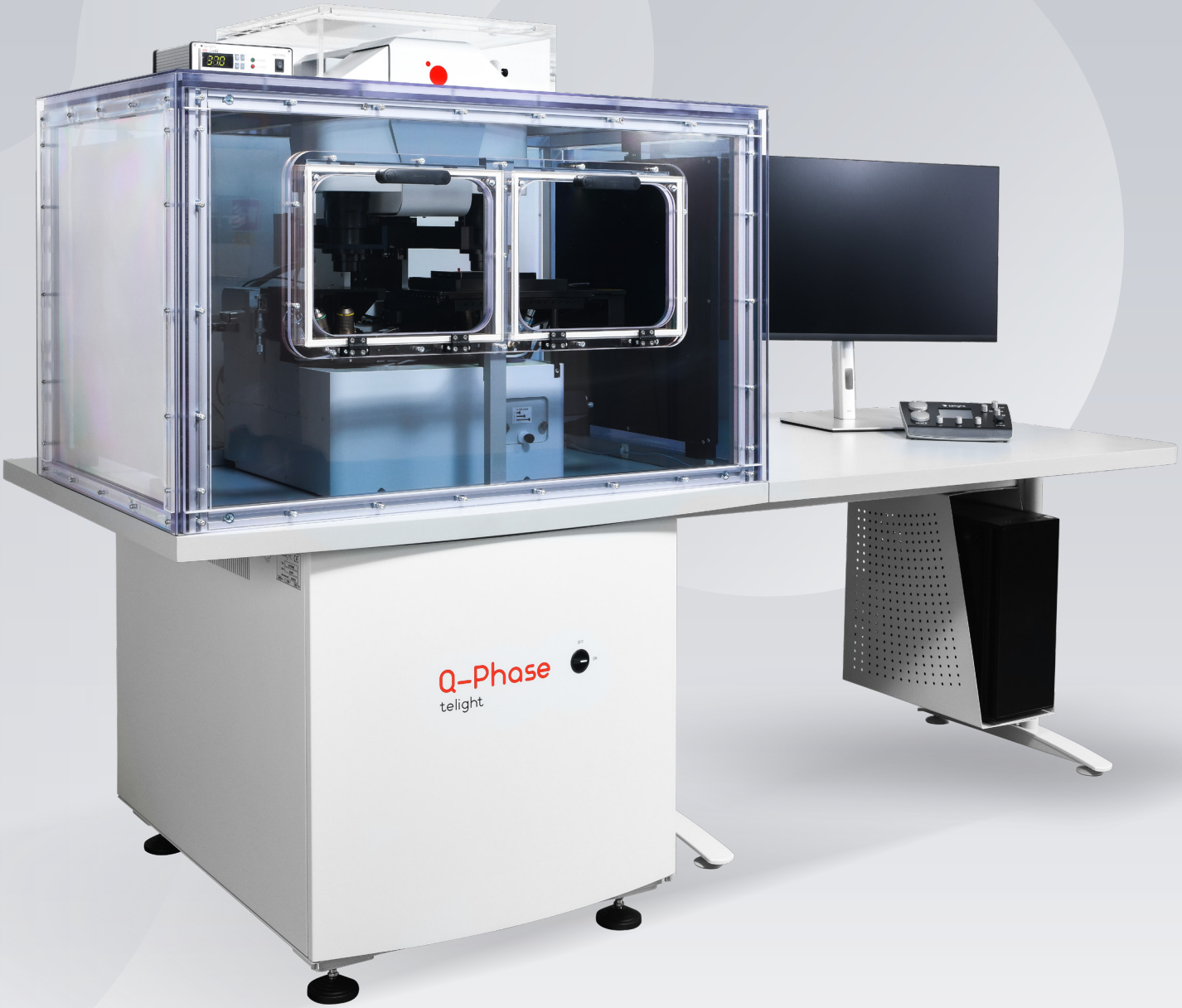
Q-Phase

Quantitative Phase Imaging

Holographic Microscope for Advanced Live-cell Analysis

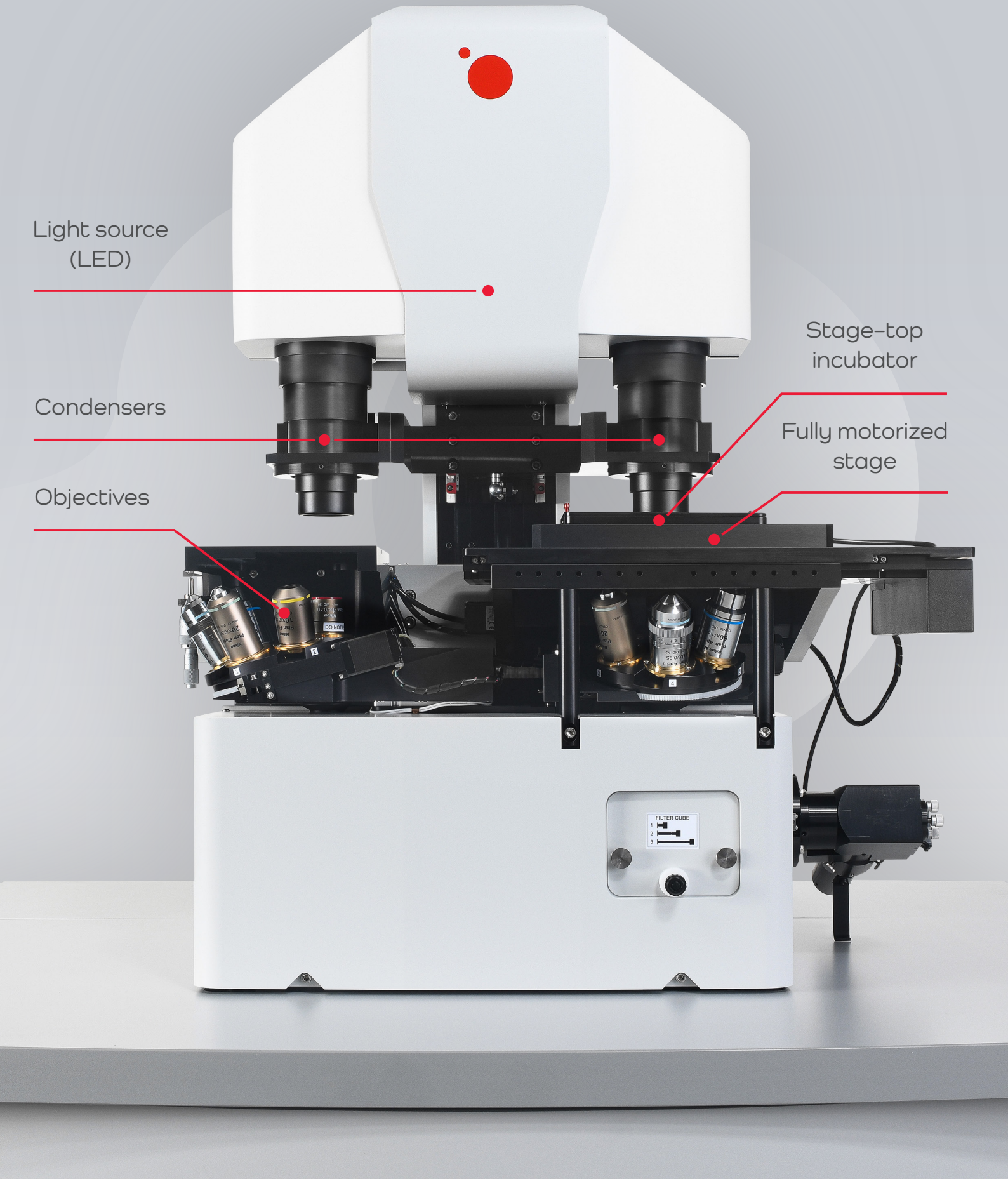


telight

telight

Explore the Unseen

Q-Phase

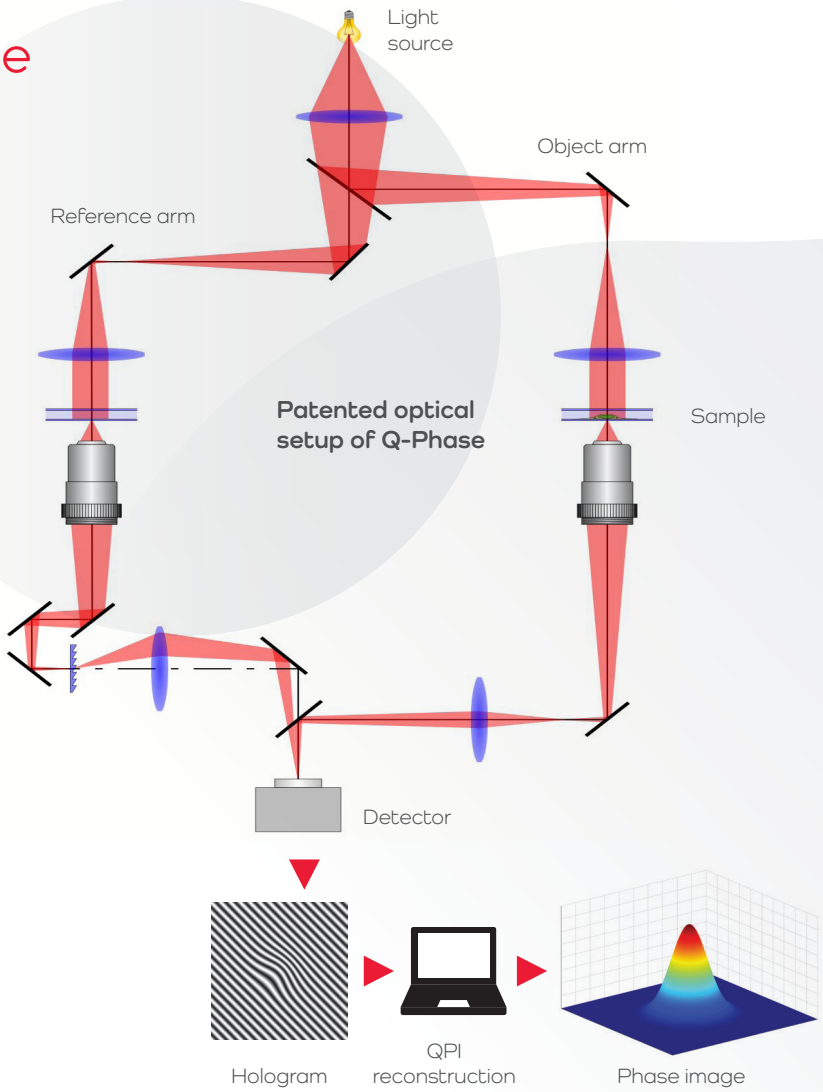

Light source
(LED)
Condensers
Objectives
Stage-top
incubator
Fully motorized
stage
FILTER CUBE

Principle behind Q-Phase

Q-Phase is based on patented technology of coherence-controlled holographic microscopy using an incoherent light source to generate high-quality images without any compromises.

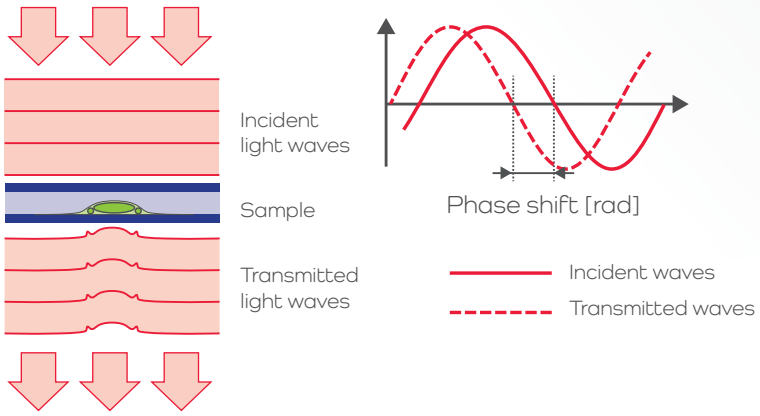
Q-Phase is an inverted transmission light microscope. The illumination light produced by an LED source is split into two separate arms. The object arm where a sample is placed and the reference arm where a reference sample (blank) is set. The beams in each arm pass through the inserted samples and are combined at the image plane of the microscope where they interfere and create a hologram. The hologram is then recorded by a detector and a quantitative phase image is extracted from the hologram in real-time by a computer.

The final output of Q-Phase is the Phase image where the light delay (phase shift) across each part of the sample is stored as a quantitative value in the corresponding image pixels. **The process of phase detection at a sample plane is referred to as Quantitative Phase Imaging (QPI).**



Quantitative Phase Imaging

The time of light propagation through a specific environment depends on the refractive index and the optical path length. **When a light wave travels through a sample with varying refractive index and/or varying height, its wavefront is distorted causing a change in the phase distribution of the wave**. The Q-Phase allows quantification of the phase distribution in the sample plane.



Precise quantification of cell dry mass (pg/µm²)

From the detected phase shift values, cell dry mass can be calculated according to the equation on the right. Q-Phase thus enables real-time measurement of cell dry mass - unique cellular parameter.

$$m = \frac{\varphi\lambda}{2\pi\alpha}$$

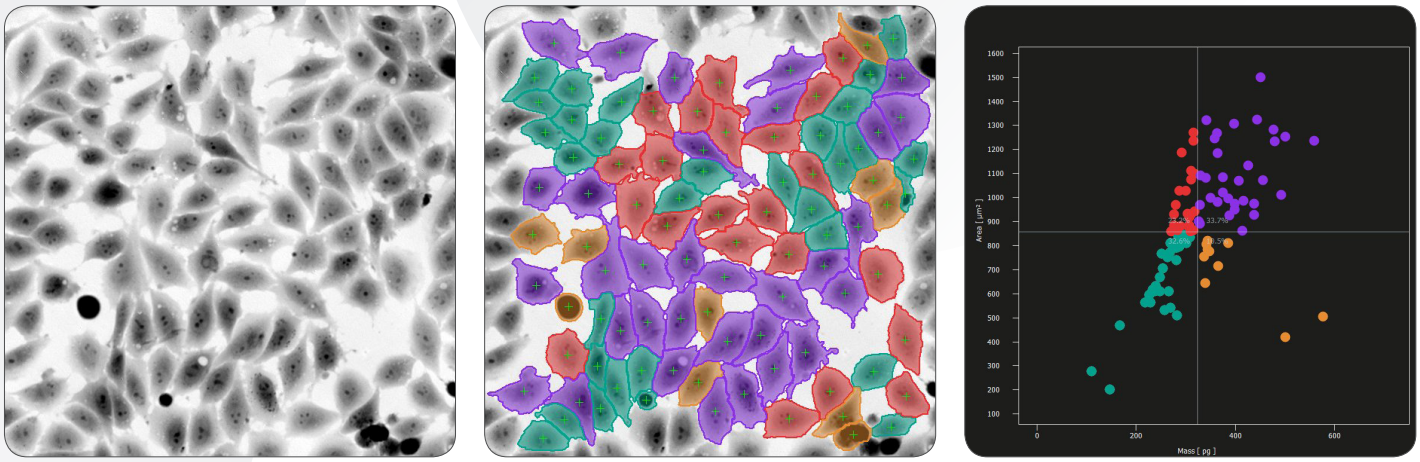
m ... cell dry mass density [pg/µm²]
φ ... detected phase value [rad] → **value detected by Q-Phase**
λ ... wavelength [µm] (λ=0.66 µm with Q-Phase)
α ... specific refraction increment = 0.18 µm³/pg

Q-Phase holographic microscope

for label-free live-cell imaging and automated cell culture analysis

Q-Phase is a holographic microscope for Quantitative Phase Imaging (QPI) designed to image cells with unmatched clarity and without the need for labeling. QPI is directly proportional to the refractive index and thickness of cells, **thus providing a high contrast image of living cells**. The Q-Phase's unique, patented QPI concept **allows accurate detection of cellular boundaries and mass changes inside cells**.

Accurate segmentation of the most transparent cell outlines can successfully be performed due to high discrimination of the image background. Furthermore, Q-Phase allows imaging of samples in scattering media - a completely novel capacity in QPI technology. Q-Phase also **provides multiple imaging modalities** such as simulated DIC (differential interference contrast), brightfield and high-pass filter imaging options in addition to fully integrated fluorescence module. Correlative imaging between the different modes is also feasible.

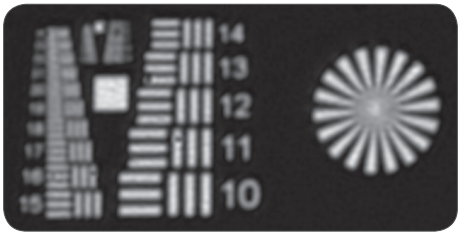


Key features:

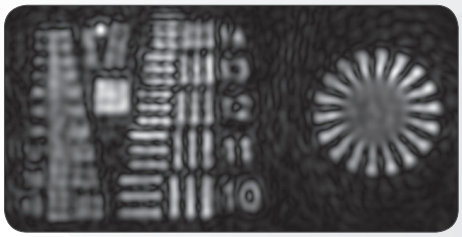
- **Live-cell imaging system:** equipped with incubator and full automation with extremely low phototoxicity for long-term studies
- **Quantitative technology:** direct measurement of cell dry mass distribution in real-time
- **Label-free approach:** non-invasive with least sample manipulation
- **Accurate cell segmentation and tracking:** well-defined background ensures accurate detection of cellular outline and thus allows image-based cell profiling
- **Multimodal imaging:** combining both QPI and fluorescence
- **Imaging in scattering matrices and turbid media**
- **High contrast and visualization of cellular compartments**
- **Comprehensive data analysis software**

Unique QPI technology

High-quality QPI is our destination. **We use an incoherent light source to eliminate the frequent optical artifact produced by systems using a highly coherent light source (laser)**. Quantitative data obtained by Q-Phase are used for direct quantification of cell dry mass changes in real-time. Thanks to the high sensitivity, even the slightest mass changes can be detected and quantified.

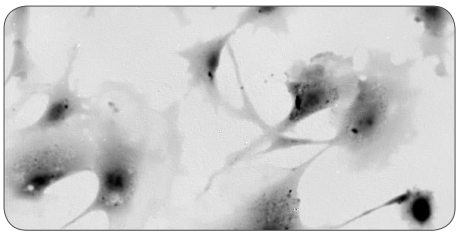


QPI with LED



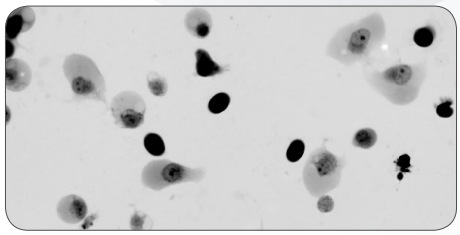
QPI with laser

A highly coherent light source (laser) is associated with lower lateral resolution and various background artifacts such as coherence noise and diffraction stripes and rings. These drawbacks can be overcome by using spatially incoherent light such as an LED.



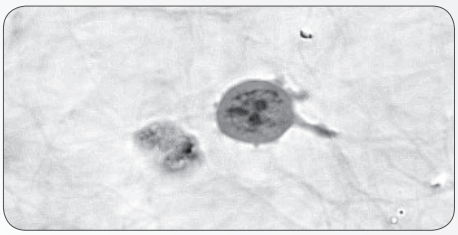
Transparent cell made visible

QPI can detect even the slightest changes in cellular mass, thus even the most transparent cells and their delicate parts can be distinguished from the background.



Cell dry mass mapping

The QPI's extraordinary real-time mass detection allows quantification of mass changes in internal parts of the cells, without the aid of specific markers.

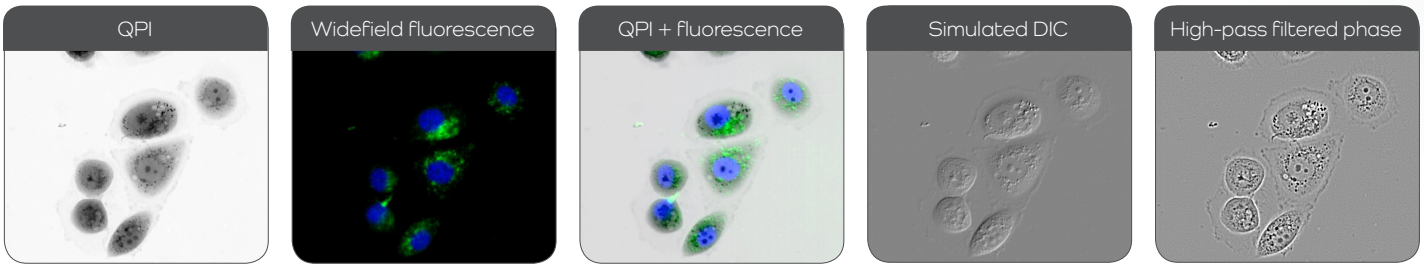


QPI in scattering media

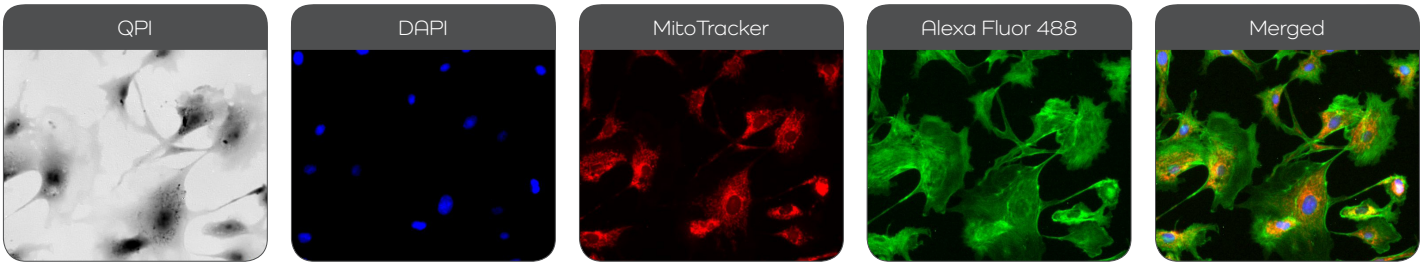
Coherence gating effect: Unique feature of the Q-Phase enables performing QPI for samples in turbid media and scattering matrices such as collagen or Matrigel.

Multimodal imaging available with the Q-Phase platform

Additional imaging modalities are accessible such as **widefield fluorescence, simulated DIC, brightfield or high-pass filtered phase**. Multiple dimensions can be combined in a single experiment and automatically acquired by the Q-Phase system (time-lapse, multi-position, multi-channel, Z-stack). The system is optimized for long-term experiments with living cells.



Images of human ovarian cancer cells (A2780), Cyto-ID® Green for acidic autophagosomes detection, DAPI for staining nuclei.

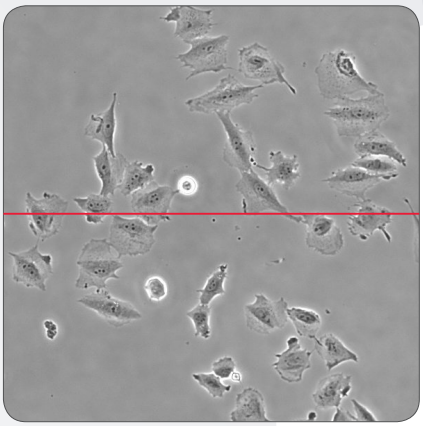


Bovine pulmonary artery endothelial cells labelled with DAPI for nuclei, MitoTracker® Red for mitochondria and Alexa Fluor® 488 for F-actin.

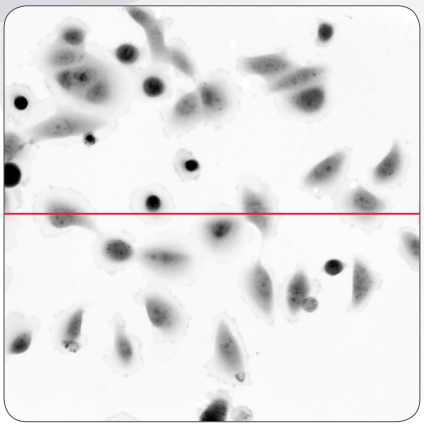
Automated label-free image segmentation

Step by step segment your time-lapse images with high accuracy

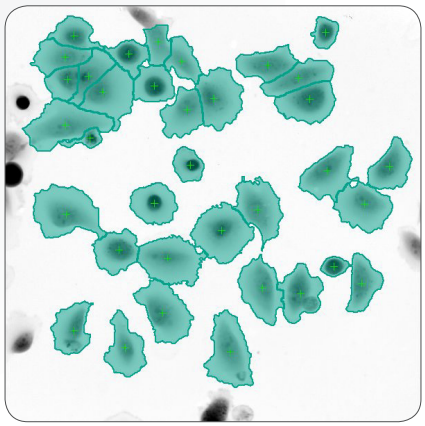
Q-Phase creates high-quality QPI data for live-cell imaging which **allows researchers not only to obtain clear contrasted images for cells but also to analyze and track them effectively**. Automatic cell segmentation represents a potent advantage in the Q-Phase system due to the precise detection of cellular boundaries and cell dry mass quantification of individual cells in large populations. QPI-based segmentation is very fast, which significantly speeds up analyses of large datasets with thousands of frames. Furthermore, **the determinant of correct segmentation is background discrimination that can be accurately distinguished in QPI technology rather than traditional light microscopy techniques**. Therefore, the more accurate segmentation, the more effective cell analysis is obtained.



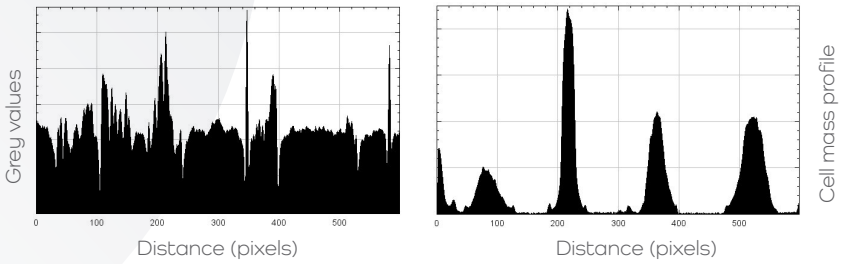
Traditional phase contrast – rat fibroblasts



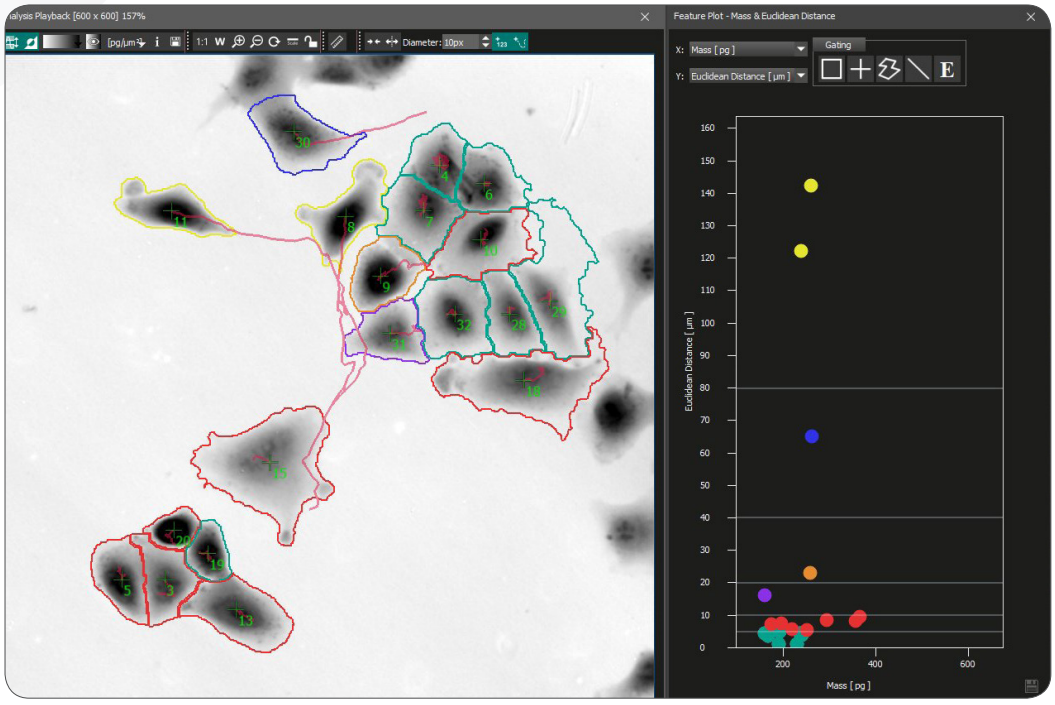
QPI (Q-Phase) - prostate cancer cells



QPI - cell segmentation



The red line crossing the cells represents the intensity values in the corresponding histograms: grey values in the phase contrast image and cell mass profile in the phase image. Optical artifacts such as the Halo effect and fluctuation in the background values significantly disturb the segmentation in the traditional phase contrast image compared to the QPI where the background has zero values which enable accurate identification of cell outlines.



Cell tracking and Euclidean distance analysis

SophiQ: Extract maximum information about your sample

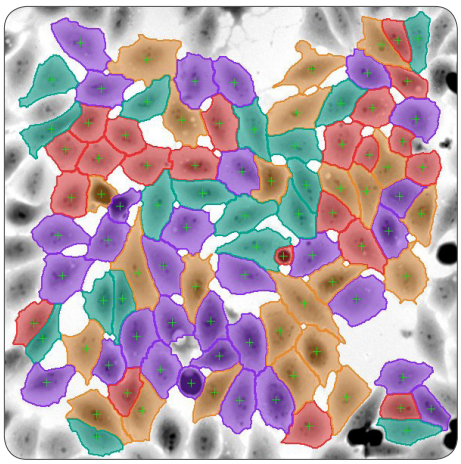
The best in class label-free image analysis software

The powerful **SophiQ analysis toolbox** processes segmented images on-the-fly and provides a complex portfolio of tools for data visualization, subpopulation gating and multi-parameter data mining. It **links quantitative data to images and individual cells**, which makes optimizing gates and checking outliers extremely easy and efficient. The resulting data can be exported in common file formats for further processing and analysis.

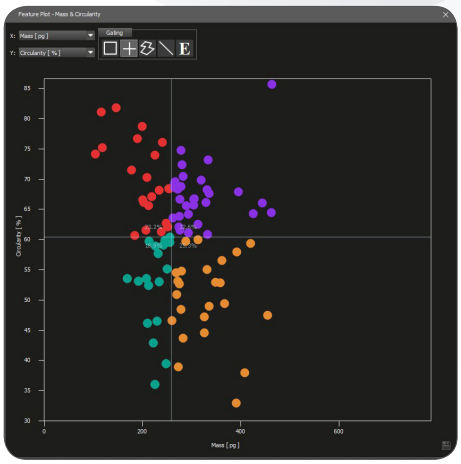
Complex portfolio of tools for data visualization, subpopulation gating and multi-parameter data mining

Parameters

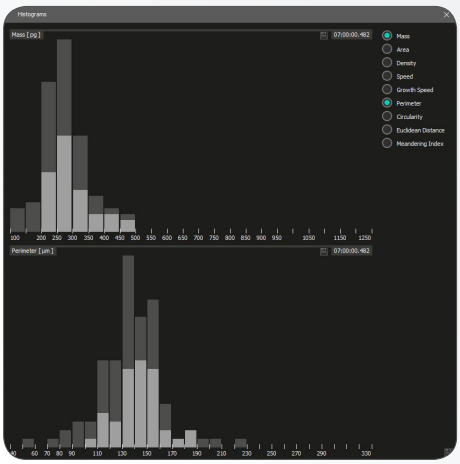
- Cell dry mass, density, area, circularity, perimeter, growth speed
- Cell speed, Euclidean distance, meandering index
- Confluence
- Fluorescence



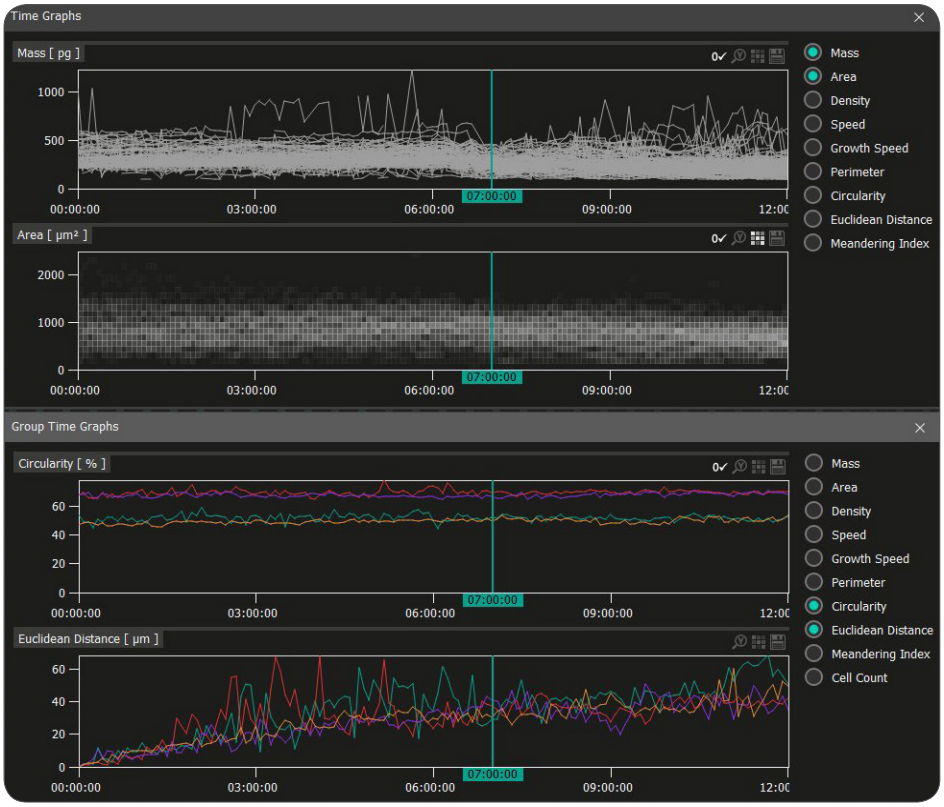
Segmentation



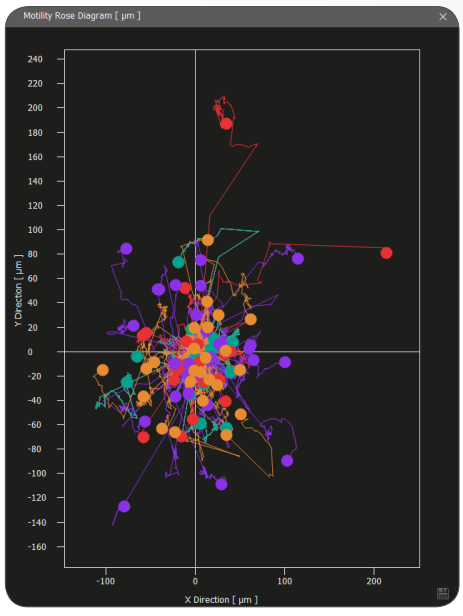
Multiple population gating



Histograms



Time graphs and heatmaps



Motility rose diagram

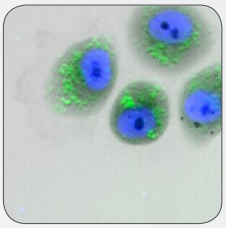
Application examples

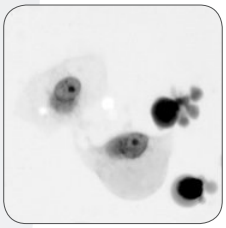
Q-Phase is aimed at applications in the area of live-cell imaging where you can quantify cell growth, morphology, dynamics as well as the changes of cell dry mass distribution in real-time. Cell dry mass serves in many aspects as a sensitive parameter for cell integrity, growth and metabolism allowing various applications in cell biology and pharmaceutical research.

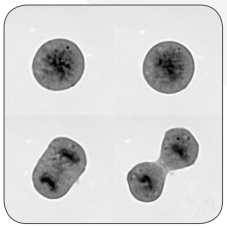
Q-Phase allows detailed assessment of cell attributes due to the extremely high sensitivity for detecting cell dry mass changes. Q-Phase provides low background noise that ensures a precise segmentation of each cell and further in-depth analysis of the cellular parameters such as cell mass, area, density, directionality, growth rate and more.

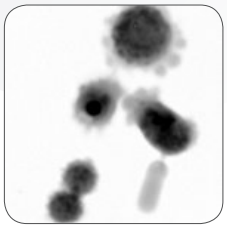
Real-time monitoring of these parameters together with the flexibility in acquiring multi-position and multimodal imaging enrich the data mining from a single experiment. Rare biological events and cells with unique behavior can be then identified in large populations of cancer cells and eventually uncover the hidden phenomena related to cancer growth and resistance.

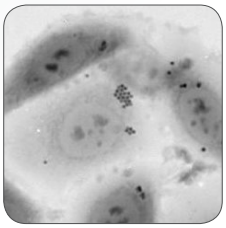
- Quantification of morphological cellular parameters
- Monitoring of cell dry mass and growth rate
- Trajectory distance and cell speed evaluation
- Detection of unique cellular events and fate

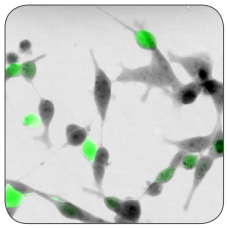

Cancer research

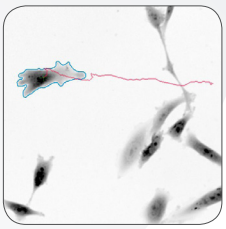

Cell death

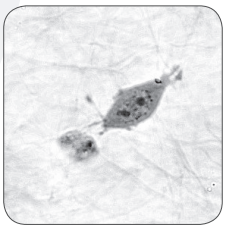

Cell cycle

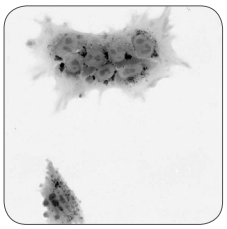

Cell morphology

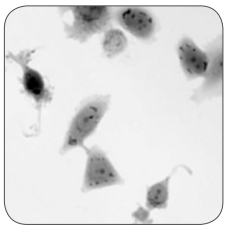

Drug toxicity

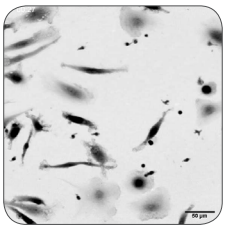

Virology

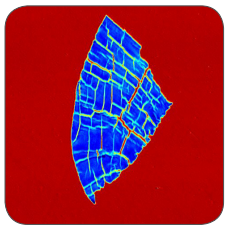

Migration studies


Extracellular matrix


Stem cells

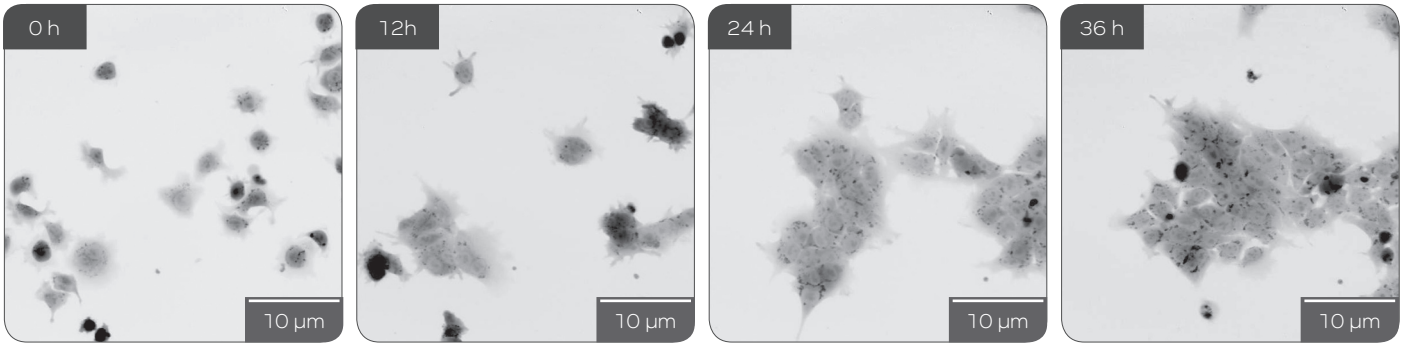

Cell interaction


Immunology


Refractive index

Stem cell research: Long time-lapse, label-free QPI

The ability of stem cells to differentiate into specialized cell types presents many opportunities for regenerative medicine, stem cell therapy and developmental biology. However, traditional methods in stem cells research are destructive, time-consuming and logistically intensive. **Q-Phase enables a non-invasive, label-free approach to study cell differentiation and provides a rapid, high-content characterization of cell and tissue cultures**.

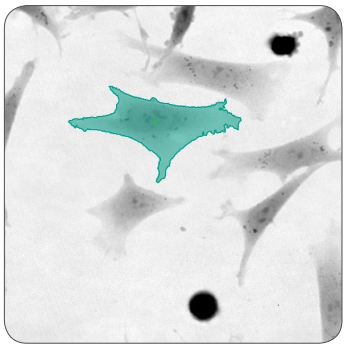


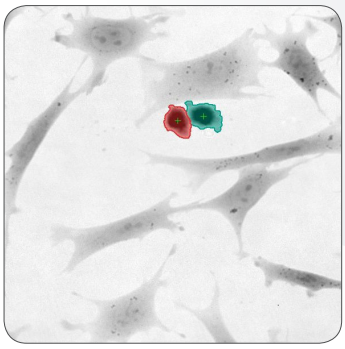
Time-lapse differentiation of human embryonic stem cells. Samples provided by Dr. Jaros, Faculty of Medicine, Masaryk University, Brno.

Monitoring of cellular growth during the cell cycle

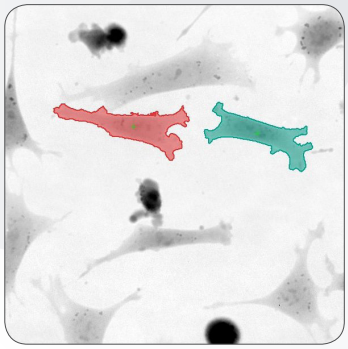
Progression through the cell cycle is one of the most fundamental features of cells. Q-Phase is a label-free quantitative tool for precise detection of the different effects elicited by drugs, chemical agents, growth factors and media on cellular growth along the cell cycle. **Single experiment using Q-Phase provides a lot of information about the cell cycle of each cell** which helps to identify symmetrical/asymmetrical cell division, cell cycle inhibition, doubling time or to detect a subpopulation that can escape the chemotherapeutic cytotoxic effect.

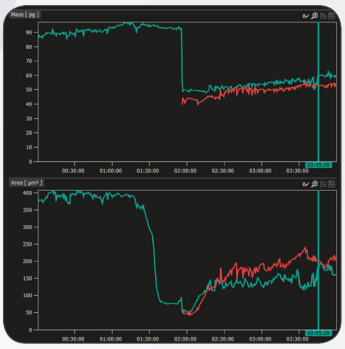
Symmetrical division





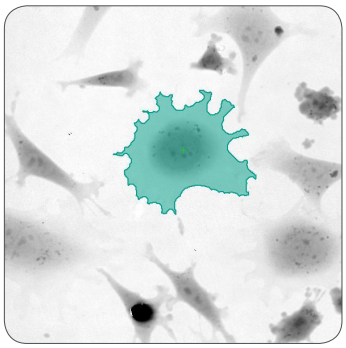
The selected cell divides symmetrically into two daughter cells with equal cell mass as shown in the SophiQ software.

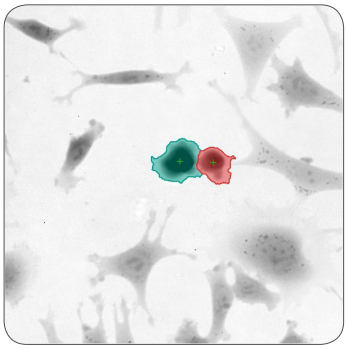




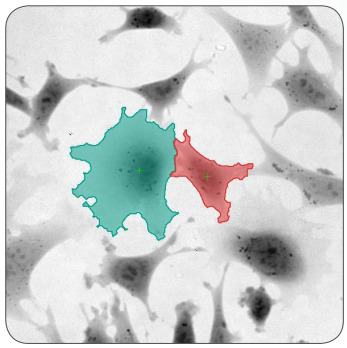
Upper plot: time graph for cell dry mass in pg. Lower plot: time graph for cell area in μm^2. The higher values at the beginning correspond to the parent cell, the lower values characterize the daughter cells.

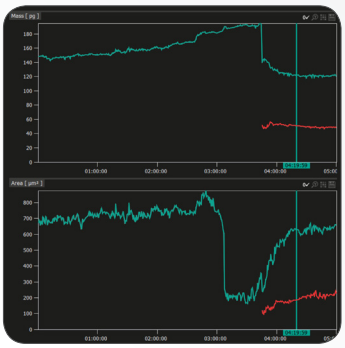
Asymmetrical division





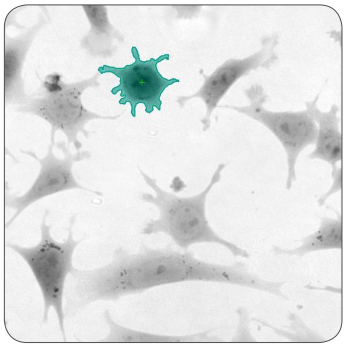
Asymmetrical cell division is a unique feature of stem cells. The selected cell divides asymmetrically into two daughter cells. Quantification of cell dry mass is shown in time graphs which confirm the asymmetrical division.

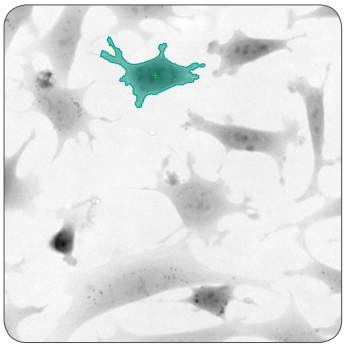


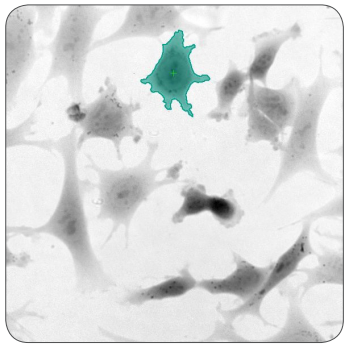


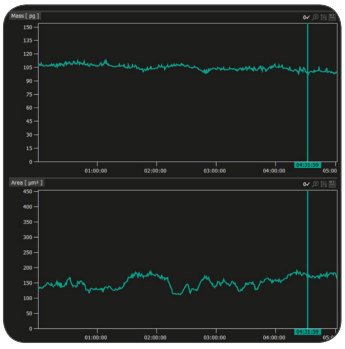
The first daughter cell is identical to the original cell in initial dry mass and cell area. The second daughter cell has a lower cell dry mass value and smaller cell area.

Cell cycle inhibition





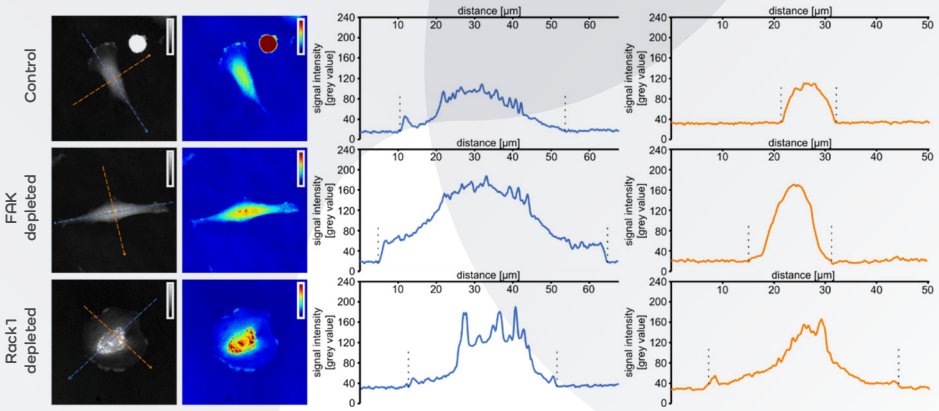




The selected cell shows a flat cell dry mass curve (upper graph) that is almost at a fixed value in all time points which indicates a quiescent cell, however, cell morphology varies during the time-lapse as shown in the lower graph displaying the fluctuations in the cell area.

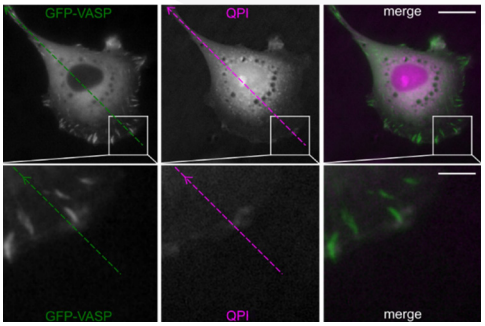
Study of cell morphology and dry mass distribution after knockdown of migratory molecules

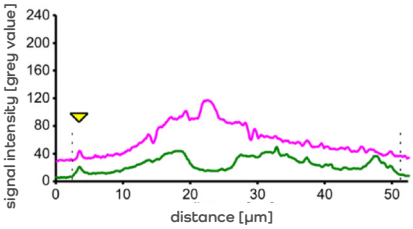
Q-Phase high phase detection sensitivity ensures the quantification of even very small changes in cell dry mass distribution whether in the central region or peripheral cell edges. Real-time assessment of cell dry mass distribution together with quantification of cell morphology and cell dynamics open the doors to study the role of adhesion and migration molecules. These molecules are involved in the cell movement and orientation, in the establishment of migratory polarity, the cell rear determination/stabilization and much more.



Depletion of the two proteins (FAK and Rack1) affects cell shape and dry mass distribution. Figures show grayscale and pseudo-colored QPI of the control cells, FAK-depleted cells and Rack1-depleted cells. FAK-depleted cells acquired bipolar phenotype while Rack1-depleted cells resulted in rounded "fried egg" phenotype with an increase in dry mass at the cell edges (cell protrusions). The graphs show a distribution of cell dry mass of corresponding diagonal (blue) and cross (orange) sections. Cell borders are indicated by dashed lines.

GFP-VASP (vasodilator-stimulated phosphoprotein) **localization and its superposition with QPI of fibroblast**. The lower panel displays a higher magnification of the boxed area. The graph shows cell dry mass distribution (purple line) and GFP-VASP intensity along the imaged cell. The yellow arrowhead indicates the increase in cell dry mass and GFP-VASP signal at the leading edge. Cell borders are indicated by dashed lines.

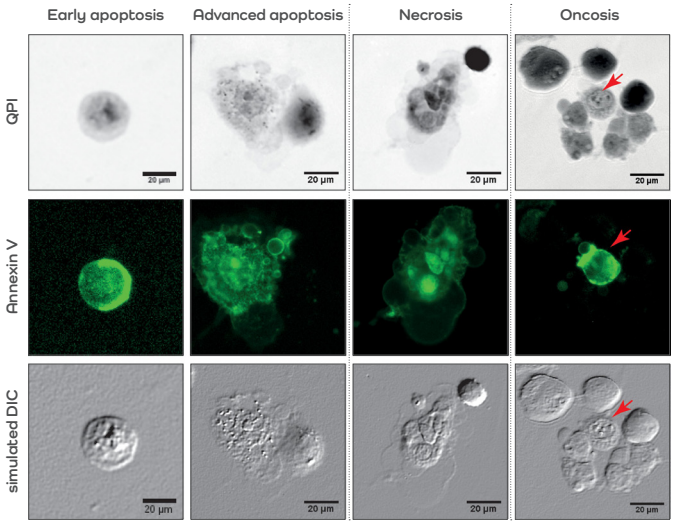




O. Ramaniuk, et al. Quantitative Phase Imaging of Spreading Fibroblasts Identifies the Role of Focal Adhesion Kinase in the Stabilization of the Cell Rear. Biomolecules 10.8 (2020): 1089.

Cellular morphology of cell death types

Despite that predominant types of cell death can be detected by flow cytometry, the absence of cellular morphology analysis leads to the underestimation and misclassification of less common cell deaths such as oncosis. The Q-Phase coupled with fluorescence detection makes it possible to distinguish oncosis clearly from other cell death types. **The real-time monitoring enables observation of particular cell death phases including the final fate of cells after the treatment,** which is a significant advantage as compared to the common light microscopy providing only a kind of the end-point analysis.



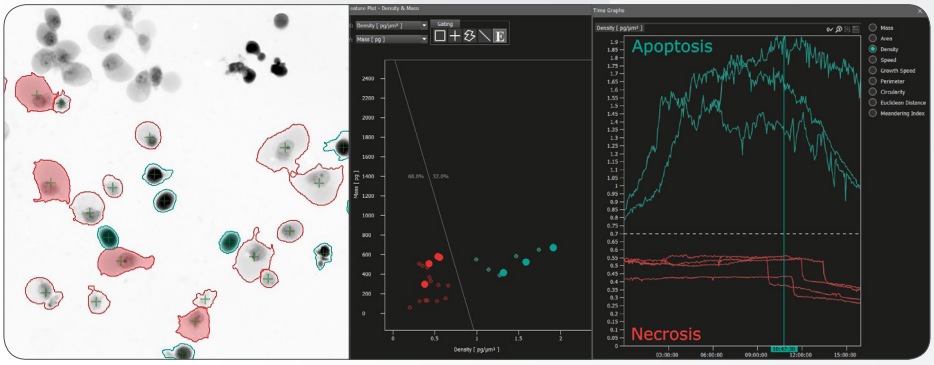
Characteristic apoptotic, necrotic and oncotic PC-3 cells in the Q-Phase with fluorescence detection and simulated DIC. Annexin V staining for the verification of cell membrane alteration. The red arrow indicates annexin V-positive "advanced" oncotic cell.

J. Balvan, et al.: Multimodal Holographic Microscopy: Distinction between Apoptosis and Oncosis, PloS One 10.3 (2015): e0121674.

Identification of specific cell death

Cell viability and cytotoxicity assays are essential for screening antineoplastic or other therapeutic drugs. Even though biochemical-based tests represent the leading method to obtain preliminary preview, their results focus on a single-point measurement whereas the overall changes during cell cultivation are usually ignored. **The morphological distinction between apoptosis and primary lytic cell death can be automatically identified through detection of dynamic time-dependent morphological changes** (shrinkage and dance of death typical for apoptosis versus swelling and membrane rupture typical for all kinds of necrosis) **and quantitative phase-based parameters such as cell density**. Thus, Q-Phase offers a precise method for rapid label-free assessment of cell death type.

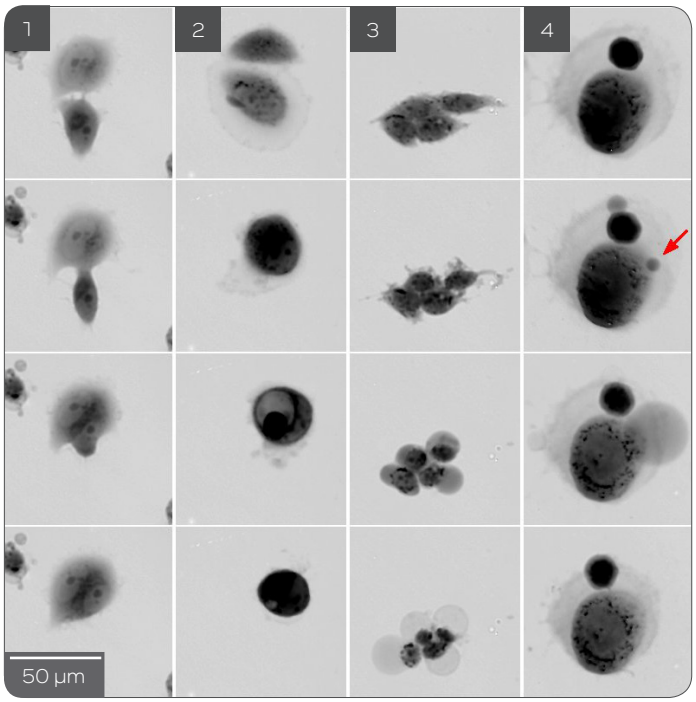
The figure shows label-free population gating of apoptotic cells vs necrotic cells using cell density parameter (cell mass/cell area). The last graph on the right shows the average value of cell density for apoptotic cells (turquoise line) vs necrotic cells (red line). The middle plot shows the gating of both populations regarding density and cell mass. Prostate cancer cell line LNCaP, 10× magnification.



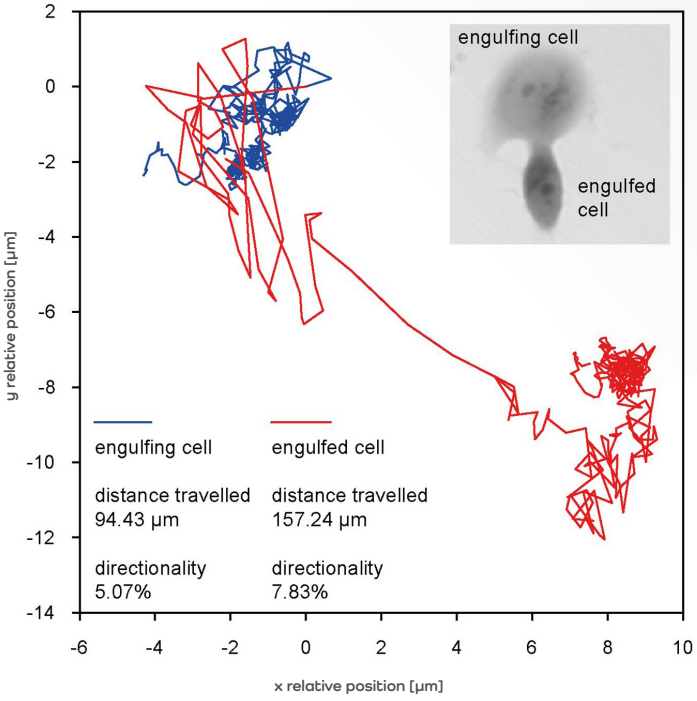
T. Vicar, et al. The Quantitative-Phase Dynamics of Apoptosis and Lytic Cell Death. Scientific reports 10.1 (2020): 1-12.

Time-lapse QPI of cell interactions

Resistant cancer phenotype is a key obstacle in the successful therapy of prostate cancer. Many different types of cell-cell interactions were recorded during the 48 hours time-lapse experiment by Q-Phase including entosis, vesicular transfer, eating of dead or dying cells, and engulfment and cannibalism of living cells.



(1) QPI of entosis; (2) QPI of cell fusion with cannibalism; (3) QPI of oncosis; (4) QPI of reverse oncosis; initial formation of the oncotic bleb (see red arrow) did not lead to necrosis.

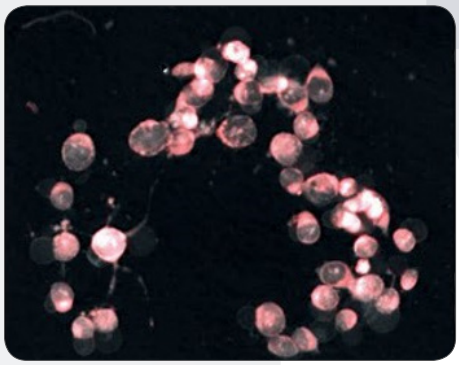


Mechanistic characterization of engulfed and engulfing cells in entosis. Trajectory traveled of both engulfing and engulfed cell until cell fusion.

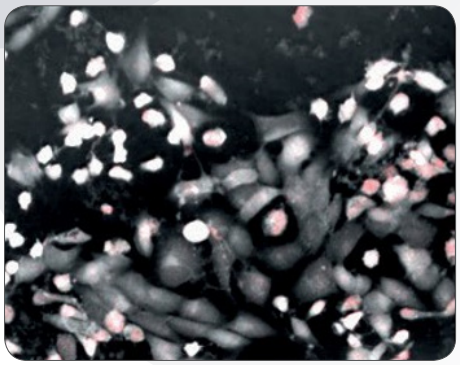
J. Balvan, et al.: Oxidative Stress Resistance in Metastatic Prostate Cancer: Renewal by Self-Eating, PLoS One 10.12 (2015): e0145016.

Elucidating the cellular uptake of drug-loaded nanocarriers

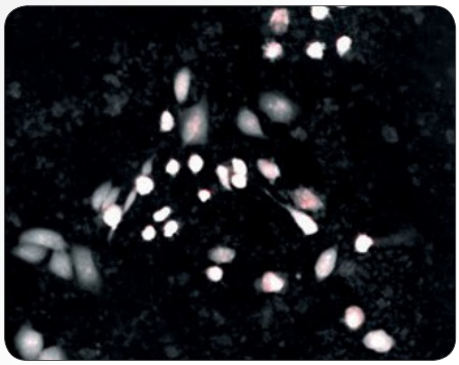
Various potent chemotherapeutic drugs have been developed over the decades. Despite their profound therapeutic efficacy, they cause numerous dose-limiting side effects, mainly systemic toxicity. To eliminate the challenges of conventional cancer chemotherapy, preferential delivery of anti-cancer drugs to tumor cells is being investigated. This can be achieved using nano-scaled drug-containing particles, which are called nanocarriers. The ideal nanocarrier needs to be not only non-toxic but also biocompatible and biodegradable and to provide easy passage through the cell membrane. For **analysis and quantification of nanocarrier internalization into tumor cells**, the quantitative phase imaging in combination with the fluorescence detection **can be easily performed by the Q-Phase microscope**. Different degrees of cellular uptake and morphological changes can be observed in cells treated with studied compounds.



Free doxorubicin



Targeted nanocarriers



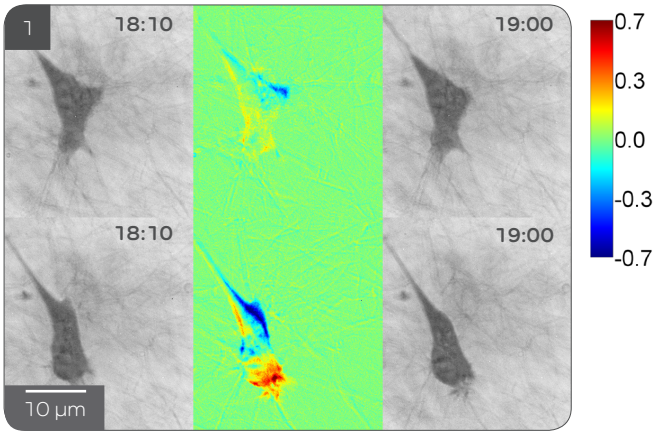
Non-targeted nanocarriers

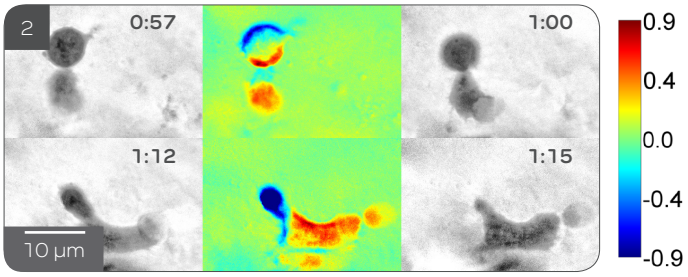
The prostate cancer-targeted and non-targeted nanocarriers were tested using subcutaneously implanted LNCaP cells and compared to free doxorubicin. Prostate cancer-targeted nanocarriers retained the high potency of doxorubicin in tumor attenuation. Figures show the morphological changes of LNCaP cells in the quantitative phase images and the uptake of doxorubicin in the red fluorescence channel.

S. Dostalova, et al.: Prostate-Specific Membrane Antigen-Targeted Site-Directed Antibody-Conjugated Apoferritin Nanovehicle Favorably Influences In Vivo Side Effects of Doxorubicin, Scientific Reports 8.1 (2018): 1-13.

Acquiring QPI for cells in 3D matrix and through scattering environment

Observation and analysis of cancer cell behavior in a 3D environment are essential for a full understanding of the mechanisms of tumor invasiveness and metastasis. However, obtaining QPI in such a cultivation environment is not possible by common QPI methods. **The coherence-gating effect, a special feature enabled by Q-Phase, makes it possible to study the dynamics of cancer cells even in a scattering milieu such as the 3D collagen matrix**. Moreover, the changes in cell dry mass distribution in migrating cells can be analyzed precisely.



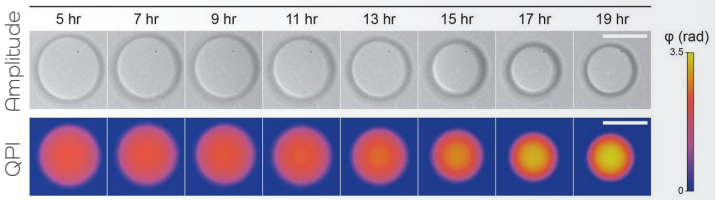


O. Tolde, et al.: Quantitative phase imaging unravels new insight into dynamics of mesenchymal and amoeboid cancer cell invasion, Scientific Reports 8.1 (2018): 1-13.

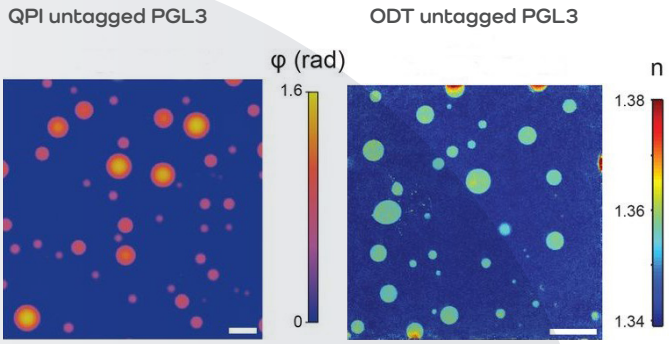
An example of cell dry mass distribution analysis is in the figures. The colored images are the subtraction of previous and following images and show the movement of cell dry mass. Areas, where the mass increased are in red, and areas, where the mass decreased, are in blue, calibration bar in pg/µm^2. Figure 1 depicts polarized cell dry mass distribution in the motile mesenchymal cell whereas figure 2 shows unequal cell dry mass distribution during the invasion of a cell through a narrow pore until the nucleus itself is able to pass.

Biomolecular condensates such as proteins and stress granules are membrane-less compartments in eukaryotic cells that concentrate biological polymers and are thus involved in the regulation of many cellular processes. They undergo self-assembly via clustering to increase the local concentration of the assembling components.

Using Q-Phase, **a precise approach to confirm the links among the condensate composition, its physical properties (e.g. thermodynamics) and functionality of condensed bi-omolecular phases** has been introduced. This method does not rely on fluorescent tags (which can significantly alter protein phase behavior) and requires 1000× less material than traditional label-free technologies. Understanding the physical properties of condensates enable rational engi-neering of protein mutations. Selective tuning of condensate properties opens the door to directly identify their impact on downstream biological function.



Timelapse of untagged PGL3 condensate. QPI reveals that PGL3 condensate density increases with time. Scale bar 10 µm.



Comparison of concentrations measured in individual PGL3 condensates by Q-Phase (QPI) and optical diffraction tomography (ODT). Scale bar 10 µm.

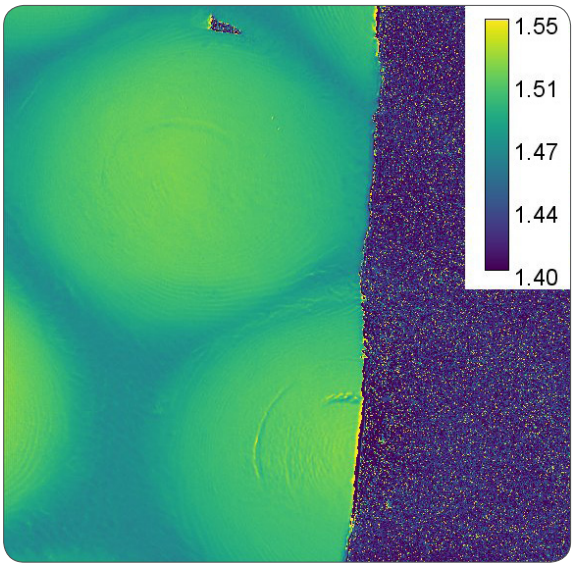
The coefficient of variation is 3× lower for Q-Phase. Moreover, Q-Phase measures the protein concentration in condensates with a 3× higher precision compared to ODT.

* PGL3 is a major component of condensates known as P granules in C. elegans.

McCall, Patrick M., et al. Quantitative phase microscopy enables precise and efficient determination of biomolecular condensate composition (2020).

Effect of structure and composition on the refractive index in biomaterials

Refractive index (RI) describes the ratio between the speed of light in a vacuum and within a material and determines the incidence and refraction angles when light passes through media of different optical densities. Moreover, it is also a key biophysical parameter that characterizes biomaterials with optical function. Thus, **studying the RI of biosensors can lead to new discoveries and reveal novel applications, as the RI plays a crucial role in designing optical setups and light guides**. An example of such biomaterial is the horseshoe crab (Limulus polyphemus) cornea, as its working principle is that of a graded RI cylinder lens.

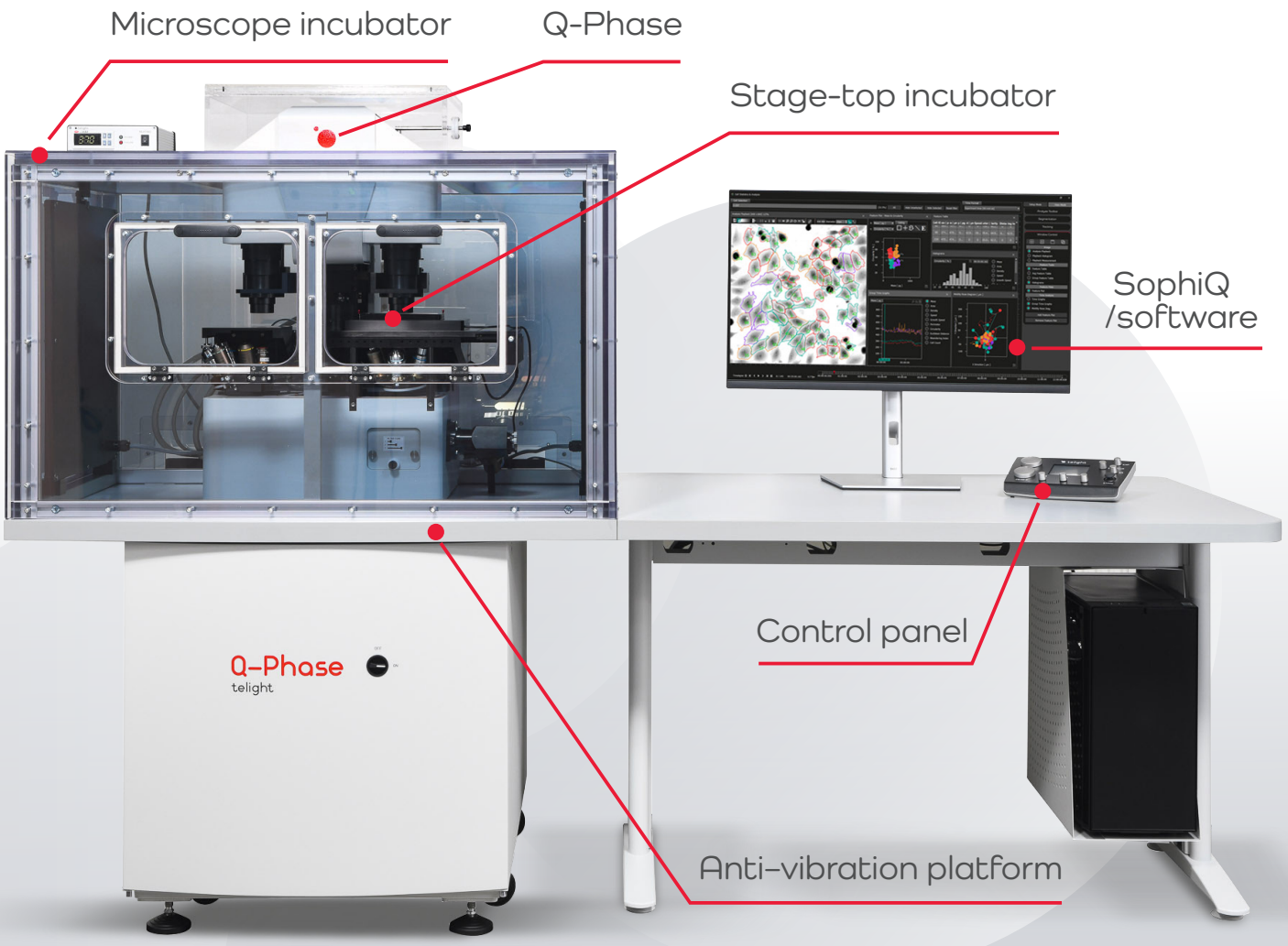


The RI of such materials can be calculated from the phase shift values measured by Q-Phase using the phase decoupling method. In this approach, the RI distribution is calculated from the difference of the phase shifts measured subsequently with the sample being surrounded by two different media with known RI.

O. Spaeker, et al. Gradients of orientation, composition and hydration of proteins for efficient light collection by the cornea of the horseshoe crab (2022).

Image of the refractive index distribution in a cross-section of Limulus polyphemus cornea. Samples provided by Oliver Spaeker, Max Planck Institute of Colloids and Interfaces, Potsdam.

Q-Phase workstation



Specifications

Light source	LED, 660 nm
Illumination power	down to 0.9 mW/cm^2
Objectives	magnification 4× to 60×
Lateral resolution	4 µm with 4× NA 0.1 objective 0.58 µm with 60× NA 1.4 objective
Field of view	objective and camera dependent, up to 1.48 mm × 1.48 mm with 4× objective
Acquisition framerate	16 fps (higher framerates on request)
Image size	1200 × 1200 px
Phase detection sensitivity	down to 0.011 rad
Accessories	control panel, microscope incubator

Optional	
Fluorescence module	LED illuminator, 3 multichannel filter cubes, motorized channel switching
Piezo-focusing	travel range 500 µm
Stage-top incubator	for precise and long-term control of temperature, humidity and CO_2 concentrations

More details about Q-Phase specifications and relevant publications can be found at our website www.telight.eu.

Q-Phase locations

- **Light Microscopy Facility**, Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany.
- **Faculty of Medicine**, Masaryk University, Brno, Czech Republic.
- **Microscopy centre Institute of Molecular Genetics AS CR**, Prague, Czech Republic.
- **Experimental Biophotonics Group**, Brno University of Technology, Brno, Czech Republic.

Video

Link

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