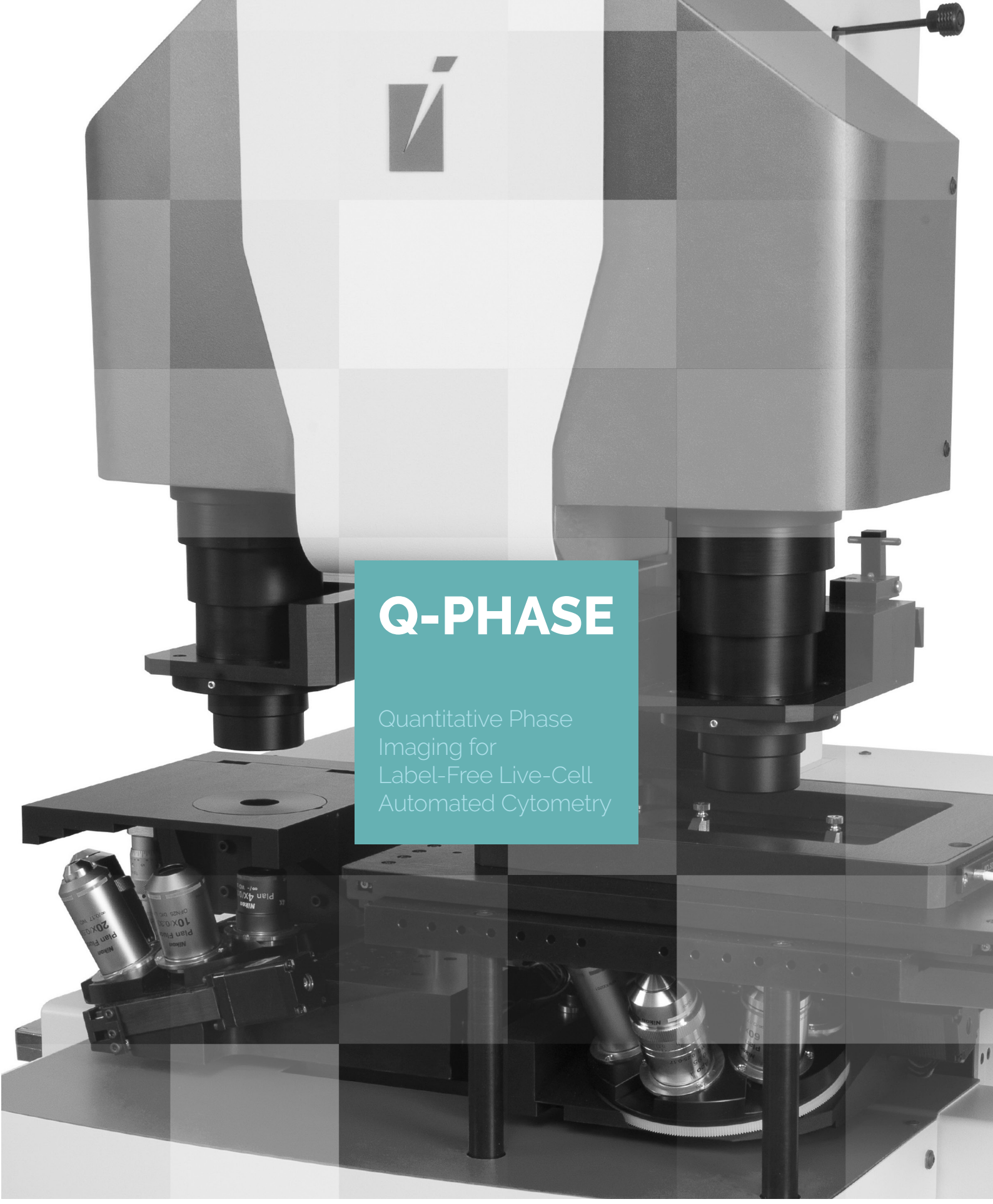


Q-PHASE

Quantitative Phase
Imaging for
Label-Free Live-Cell
Automated Cytometry

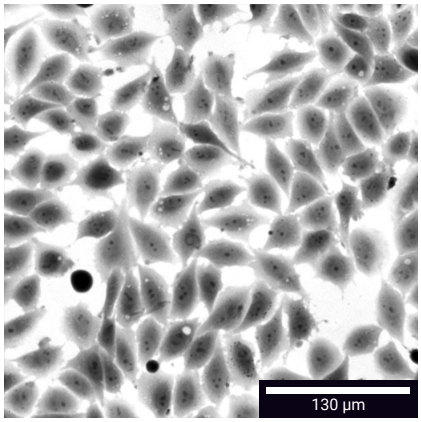
TESCAN
PERFORMANCE IN NANOSPACE

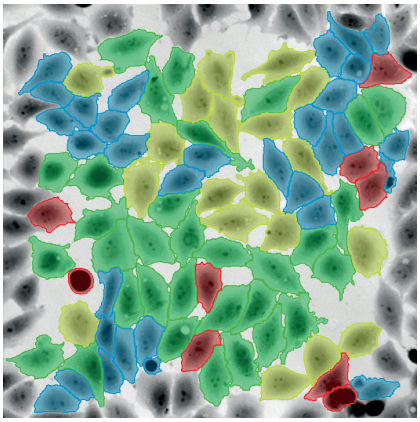
Q-PHASE holographic microscope

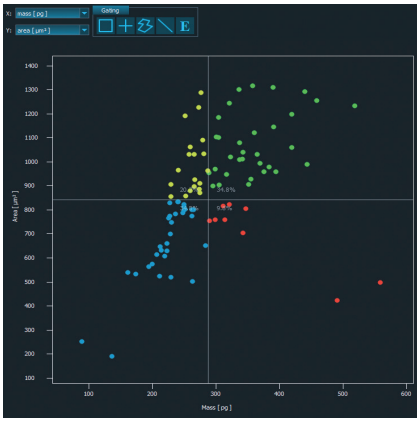
Unique holographic microscope for label-free live-cell automated cytometry

Q-PHASE is a Multimodal Holographic Microscope for Quantitative Phase Imaging (QPI) designed to image cells with unmatched clarity and without the need for labelling. QPI is directly proportional to the refractive index and thickness of cells, thus providing an unparalleled view of living cells. The Q-PHASE's **unique, patented QPI concept** allows straightforward detection of cellular boundaries and mass changes inside cells. Even the most transparent cells and their components can easily be discriminated from the background. Furthermore, it allows the imaging of samples in scattering media - **a completely novel idea in QPI imaging.** Q-PHASE enables **multiple imaging modes** with fully integrated fluorescence module, simulated DIC, brightfield and high-pass filter imaging options. Correlative imaging between the different modes is also possible.

OBSERVE › SEGMENT › ANALYZE







Key features:

- True, label-free imaging cytometry
- Low phototoxicity and fast acquisition
- High contrast detection of subcellular compartments
- Extremely sensitive visualization of cellular morphology
- Precise segmentation without the need of fluorescence labelling
- Precise cell tracking and speed measurement
- Long, time-lapse observations
- Direct quantitation of changes in cellular mass distribution
- Multimodal imaging
- Comprehensive data analysis software

Applications:

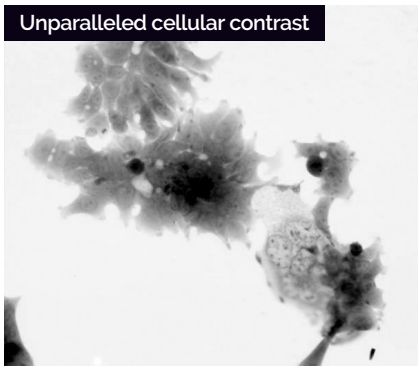
Q-PHASE is aimed at applications in the area of live cell imaging, with emphasis on cell biology, cancer research, drug testing and necrobiology; all of these, fast-developing research fields.

- **Cell biology**
  - Proliferation and differentation
  - Cell shape and volume
  - Motility
- **Synthetic Biology**
  - Coacervates
  - Transparent and phase objects observation
- **Cancer research**
  - Migration assay
  - Speed measurement
  - Cell-to-cell interaction
- **Host Pathogen interactions**
  - Virus-infected cells
  - Bacteria-cell interactions
- **Morphology**
  - Cytoskeleton organization
  - Extracellular space interactions
  - Cellular intake
- **Cells in 3D environments**
  - Tissue mimics
  - Cell invasion

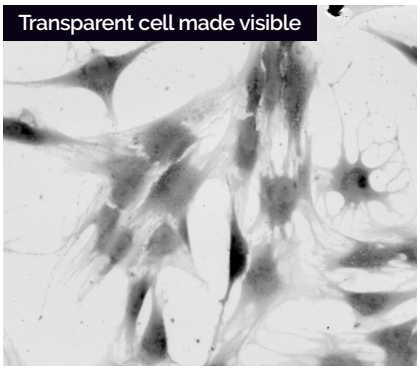
Unique QPI technology

Bringing a completely new approach to the live cell imaging

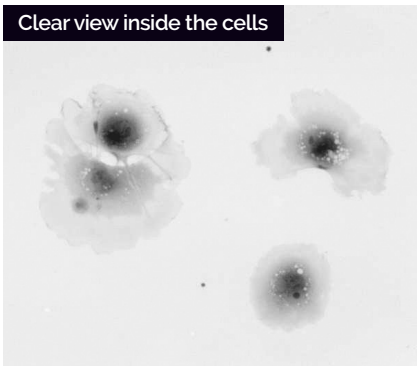
Q-PHASE uses a patented technology of coherence-controlled holographic microscopy to provide QPI of the highest quality. Quantitative data obtained by Q-PHASE are directly proportional to the cellular dry mass and can be used for monitoring and direct quantification of dry mass changes inside the cells. Thanks to the high sensitivity, even the slightest mass changes can be detected and quantified.



QPI is directly proportional to the refractive index and thickness of cells, thus providing an unparalleled view of living cells without any labelling.



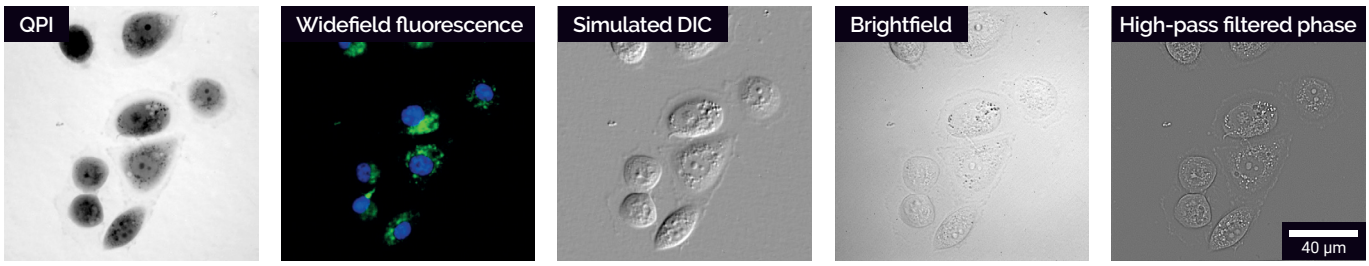
QPI can detect even the slightest changes in cellular mass, thus even the most transparent cells and their parts can be distinguished from the background.



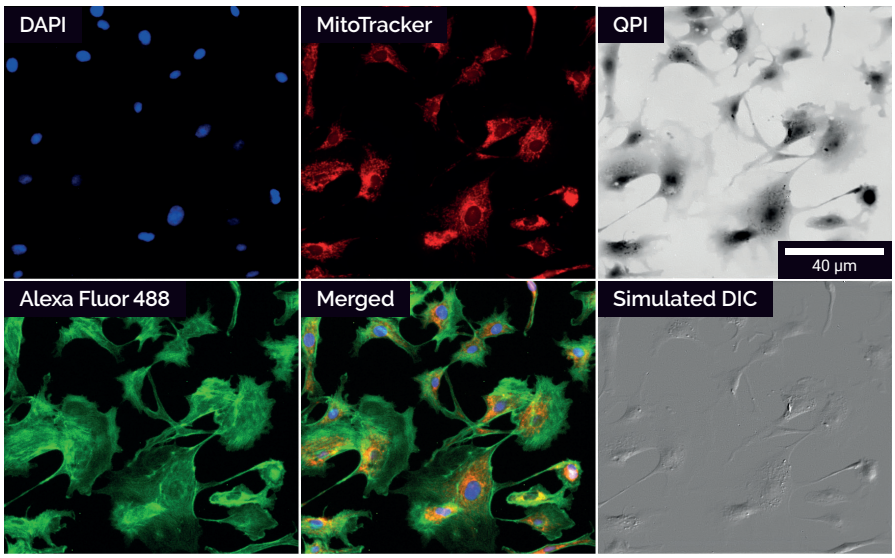
The QPI's extraordinary mass detection allows detecting changes in internal parts of the cells, such as nuclei, vacuoles, and many more, without the aid of specific markers.

Multimodal imaging available with the Q-PHASE platform

Additional imaging modalities are available 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 (timelapse, multi-position, multi-channel, Z-stack). The system is optimized for long-term several-day experiments with living cells.



▲ Images of human ovarian cancer cells (A2780), acidic autophagosomes detection



All modalities can be collected simultaneously to provide a more complete picture in a synergistic fashion

◀ Bovine pulmonary artery endothelial cells (BPAEC) labelled with DAPI for nuclei, MitoTracker® Red CMXRos for mitochondria and Alexa Fluor® 488 phalloidin for F-actin. Fluorescent images were acquired using DAPI/FITC/TRITC optical filter set.

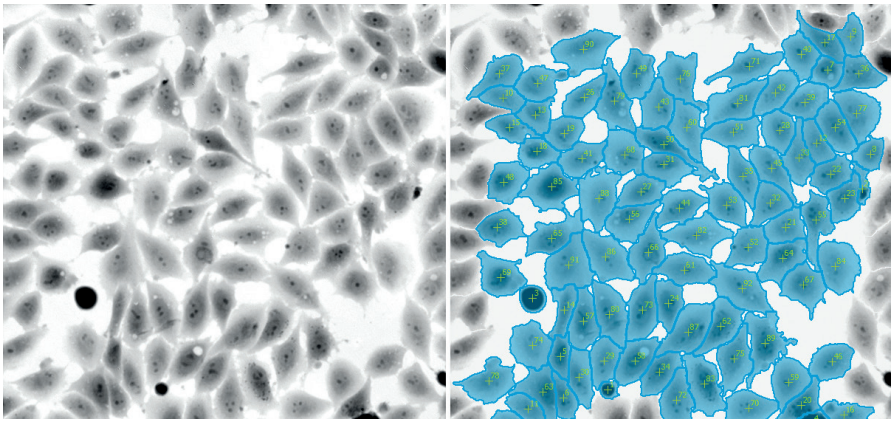
SEGMENT

Fully automated label-free image segmentation

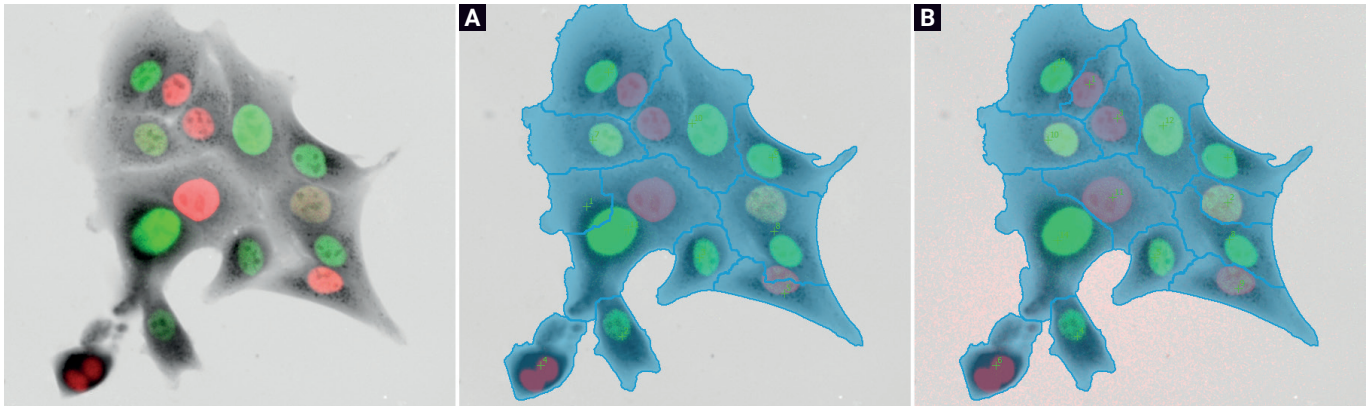
Automatically SEGMENT the data with high accuracy

High clarity QPI data allows automatic segmentation of images based on the precise detection of cellular boundaries and quantitative mass distribution of individual cells in large populations. QPI-based segmentation is very fast, which significantly speeds up analyses of large datasets with thousands of frames. Moreover, additional fluorescence data can be utilized to refine the segmentation even further.

- **QPI overcomes difficulties with segmentation of standard optical microscope images**

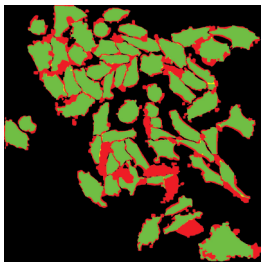
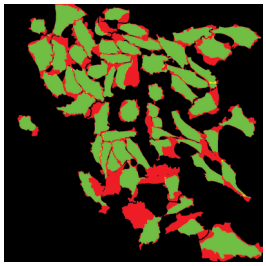
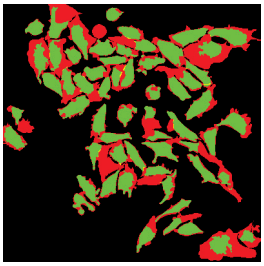
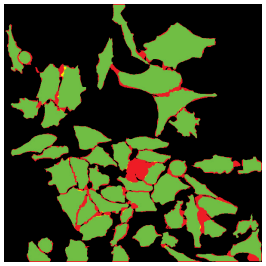


▲ PC-3 cells. Segmentation of QPI data.



▲ Fucci-expressing NMuMG cells. A. Segmentation of QPI data. B. Segmentation of QPI data corrected by nuclear fluorescence.

- **Precise cell detection and segmentation are essential factors for correct analysis of cell population kinetics**
- **QPI images offer the highest cell/background contrast among other label-free imaging techniques**

Method	DIC (algorithm 1)*	DIC (algorithm 2)**	Phase Contrast (algorithm 1)*	QPI
				
Mean Dice Index	0.71	0.66	0.61	0.84
Processing Time	Minutes	Minutes	Minutes	Seconds

▲ Correct segmentation - green, incorrect segmentation - red (compared to ground truth)

** K. Li, et al.: Nonnegative Mixed-Norm Preconditioning for Microscopy Image Segmentation, International Conference on Information Processing in Medical Imaging, Springer Berlin Heidelberg, 2009.*

*** K. Koos, et al.: DIC image reconstruction using an energy minimization framework to visualize optical path length distribution, Scientific Reports 6, 2016.*

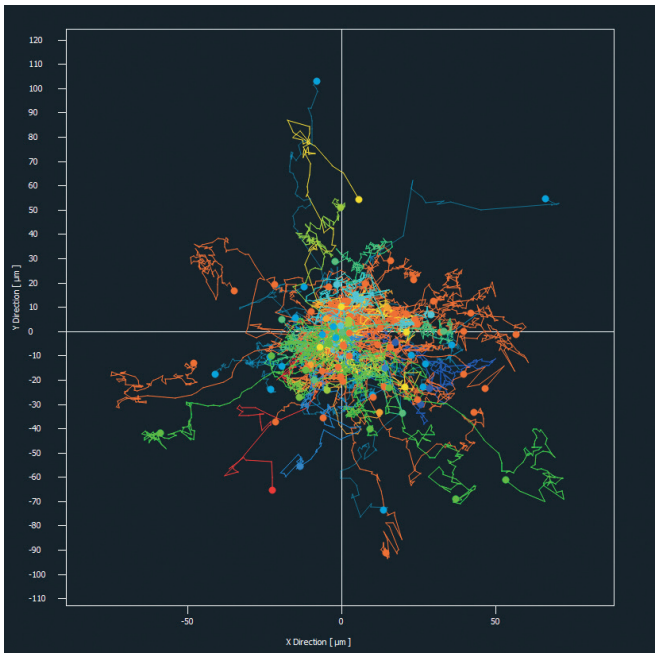
ANALYZE

The best in class label-free image analysis software

Q-PHASE Cell Analyser: Extract maximum information about your sample

The powerful Q-PHASE 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. Resulting data can be exported in common file formats for further processing and analysis.

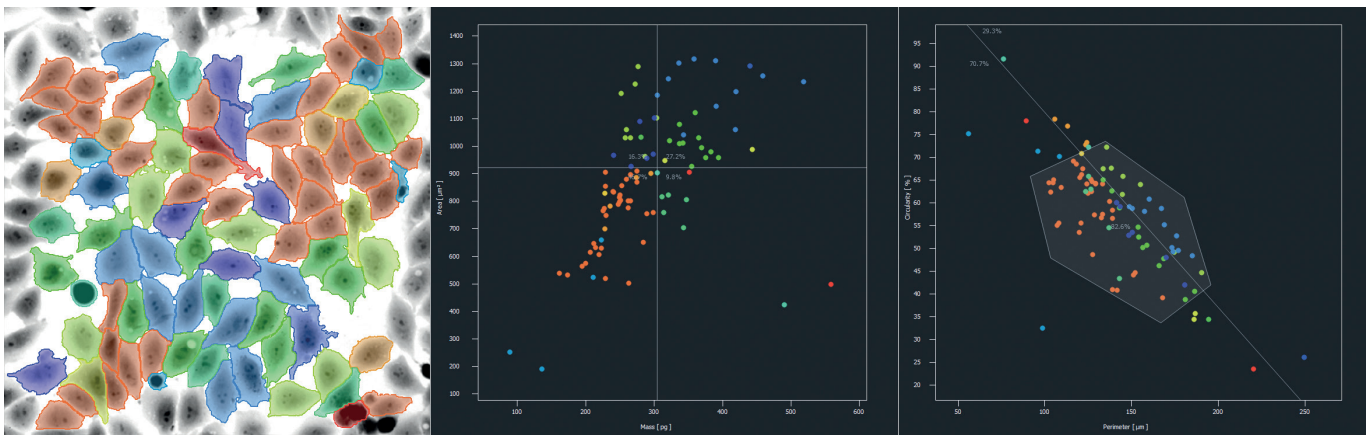
Complex portfolio of tools for data visualization, subpopulation gating and multi-parameter data mining



▲ Rose plots for motility

▲ Time graphs and heatmaps

▲ Histograms

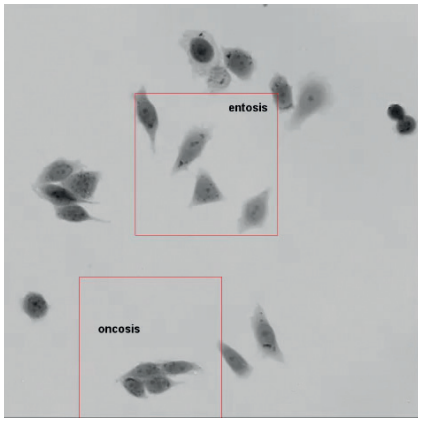


▲ Multiple population gating

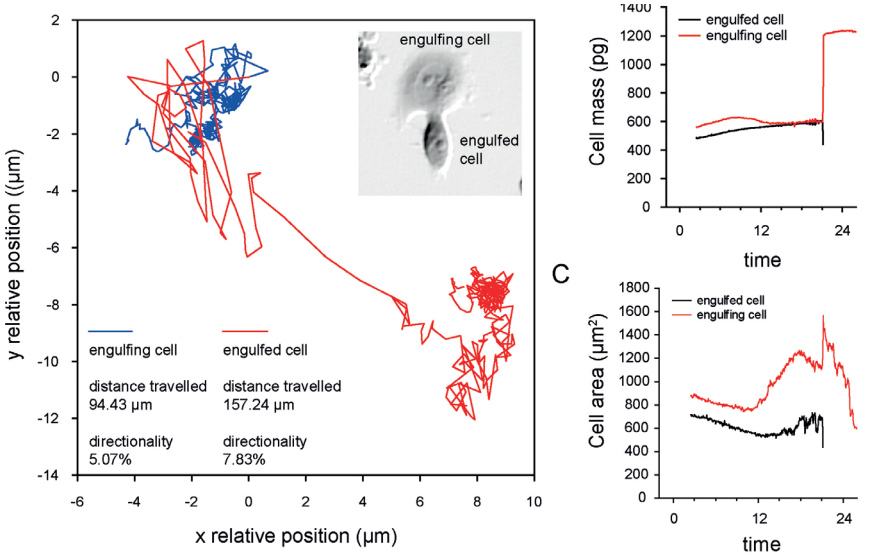
Application Examples

Cancer research

QPI allows detailed assessment of cell attributes due to the extremely high sensitivity in detecting even the smallest changes in mass density. This in turn allows very good segmentation of individual cells and further in-depth analysis of different cellular parameters such as mass changes, area, directionality, growth rate and many more. Based on these parameters, rare cells with unique behavior can be identified in large populations of cancer cells and eventually provide answers to origins of chemotherapy resistance.



- Identification of individual cell behavior
- Detection of rare cellular events
- Clear insight into various cellular processes

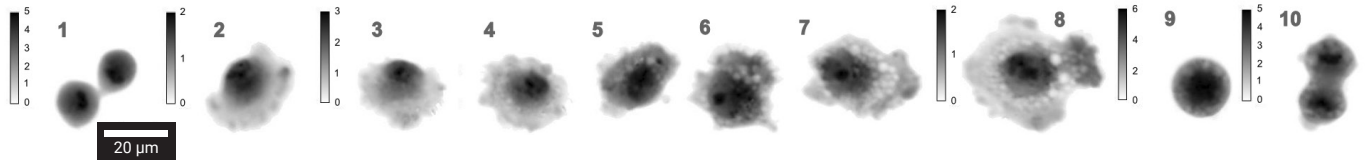


▲ Role of entosis in oxidative stress resistance of PC-3 prostate cancer cells

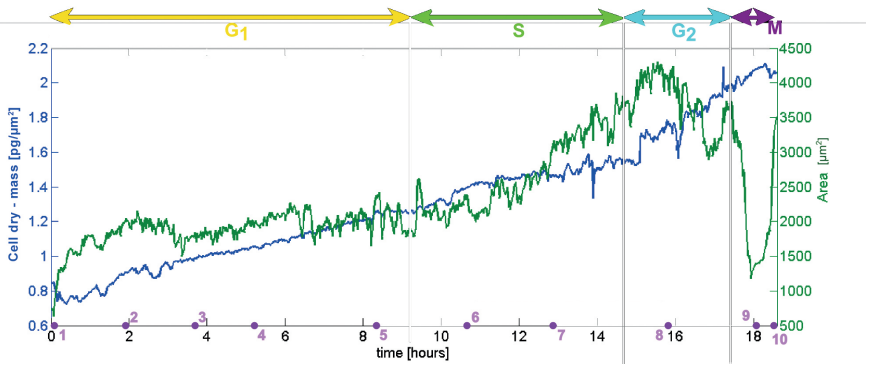
For more details see: J. Balvan, et al.: Oxidative Stress Resistance in Metastatic Prostate Cancer: Renewal by Self-Eating, PLoS One 10(12), 2015.

Monitoring of cell growth during the cell cycle

Progression through the cell cycle is one of the most fundamental features of cells. Studies of the cell cycle have traditionally relied on the analysis of populations, and they often require specific markers or the use of genetically modified systems, making it difficult to determine the cell cycle stage of individual, unperturbed cells. The unique QPI modality of Tescan Q-PHASE provides quantitative information for label-free monitoring of the cell growth as well as morphological and phenotypic changes at the single cell level.



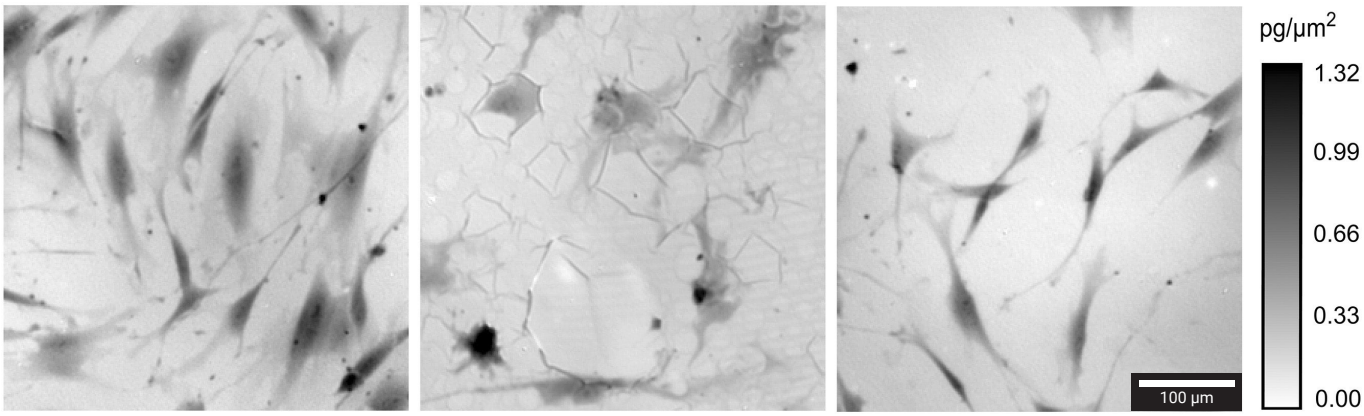
▲ QPI images illustrating cell morphology at marked out points in the life cycle of LW13K2 cell



▶ Changes in cellular mass and area during the cell cycle of LW13K2 cell. The value of mass has deen doubled between two mitosis.

Biocompatibility testing

With the tremendous increase in development of newly engineered biomaterials, there is an indisputable need for evaluations of their safety and hazard risks. Cell culture systems are often used for monitoring of biological response to the properties of new materials. The coherence-controlled holographic microscopy (CCHM) enabled by Q-PHASE provides an ideal technique for label-free monitoring of the cell-surface interaction. CCHM allows quantitative phase imaging. From such images, valuable morphological parameters of cells directly related to the cell dry mass can be extracted. Based on those parameters, viability of cells cultivated on surfaces treated with different biomaterials can be studied and evaluated.

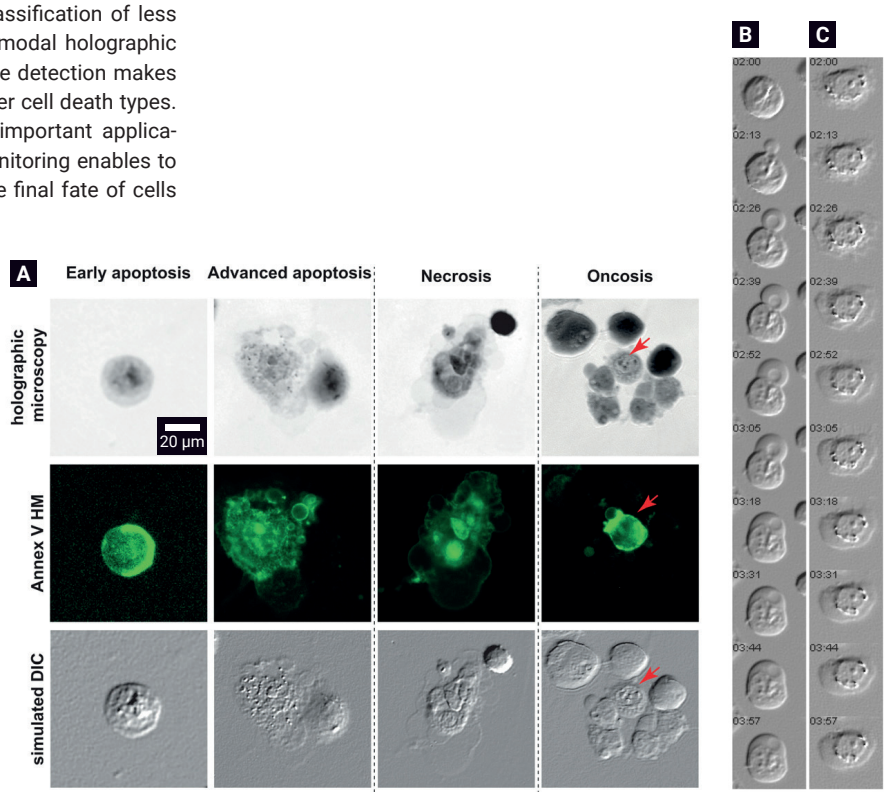


▲ Quantitative phase images obtained by CCHM. Viability of human dermal fibroblasts (LF cells) grown on the CPA40 (left) and CPA42 (middle) polymer substrates, and control (right) sample. Scale bar and calibration bar apply to the three images.

Taken from: L. Strbkova, et al.: The adhesion of normal human dermal fibroblasts to the cyclopropylamine plasma polymers studied by holographic microscopy, Surface and Coatings Technology 295, 2016.

Identification of specific cell death

When assessing the major effect of a particular therapeutic drug, it is essential to know which type of cell death is involved most in the drug response. Predominant types of cell death can be detected by flow-cytometry (FCM). Nevertheless, the absence of cellular morphology analysis leads to the underestimation and misclassification of less common cell deaths such as oncosis. The multimodal holographic microscope Q-PHASE coupled with a fluorescence detection makes it possible to distinguish oncosis clearly from other cell death types. Dynamic monitoring of treated cells is another important application of holographic microscopy. The real-time monitoring enables to observe particular cell death phases including the final fate of cells after the treatment, which is a significant advantage as compared with the common light microscopy providing only a kind of the end-point analysis.



▶ Identification of specific cell death by Q-PHASE

A) Characteristic apoptotic, necrotic and oncotic PC-3 cells in multimodal holographic microscope with fluorescence detection and simulated DIC (differential interference contrast). Annexin V staining for the verification of cell membrane alteration. Red arrow indicates annexin V-positive "advanced" oncotic cell.

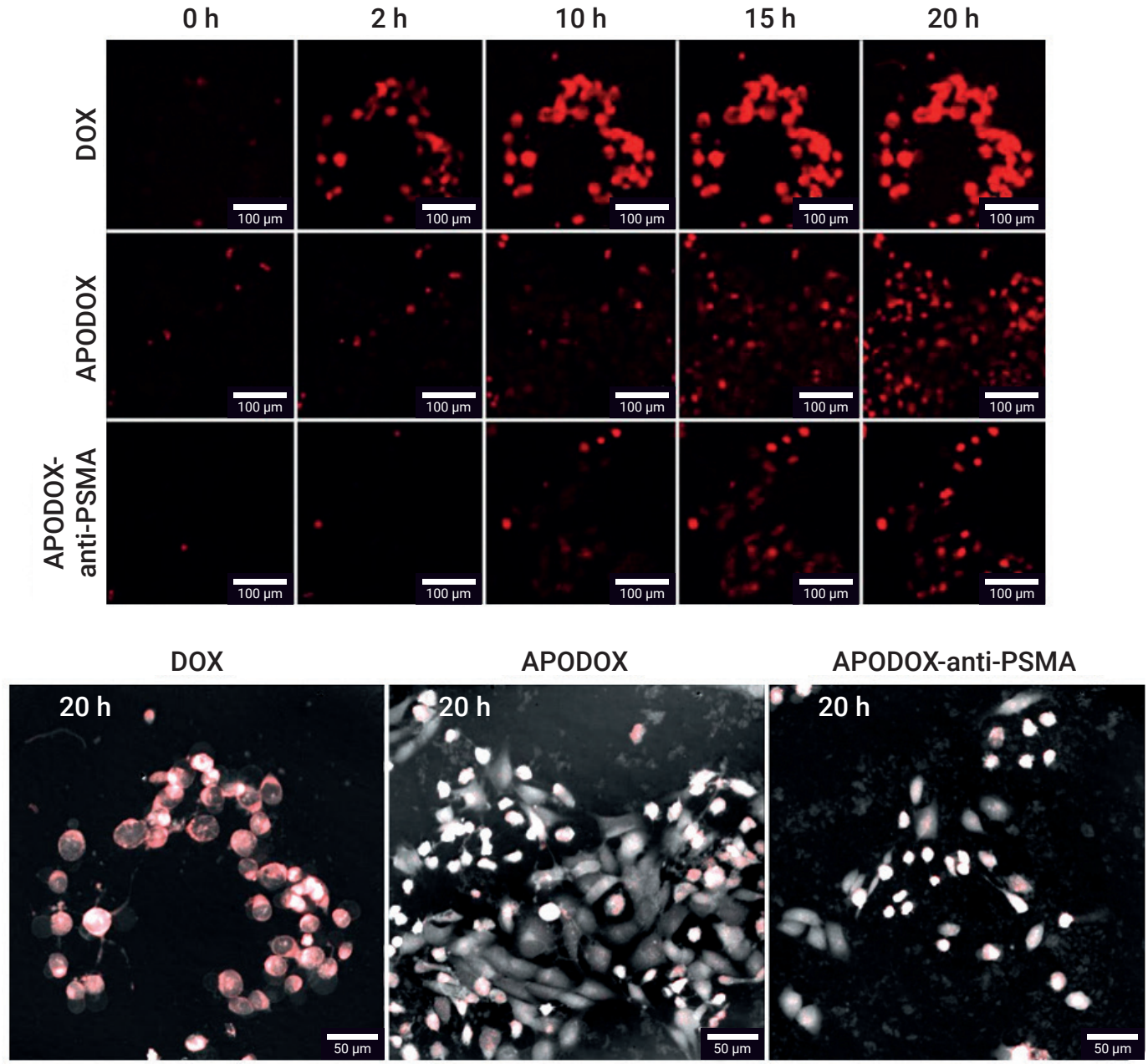
B) Time-lapse simulated DIC of typical oncotic "increase size" cell.

C) Time-lapse simulated DIC of typical "decrease size" apoptotic cell. 20 × magnification was used.

Adapted and modified from: J. Balvan, et al.: Multimodal Holographic Microscopy: Distinction between Apoptosis and Oncosis, PloS One 10(3), 2015.

Elucidating the cellular uptake of drug loaded nanocarriers

Various potent chemotherapeutic drugs have been developing 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 not only be non-toxic but also biocompatible and biodegradable and provide easy passage through the cell membranes. For analysis and quantitation of nanocarrier internalization into tumor cells, the quantitative phase imaging in combination with the fluorescence detection can be easily performed by the Tescan Q-PHASE microscope. Different degrees of cellular uptake and morphological changes can be observed in cells treated with targeted compounds.

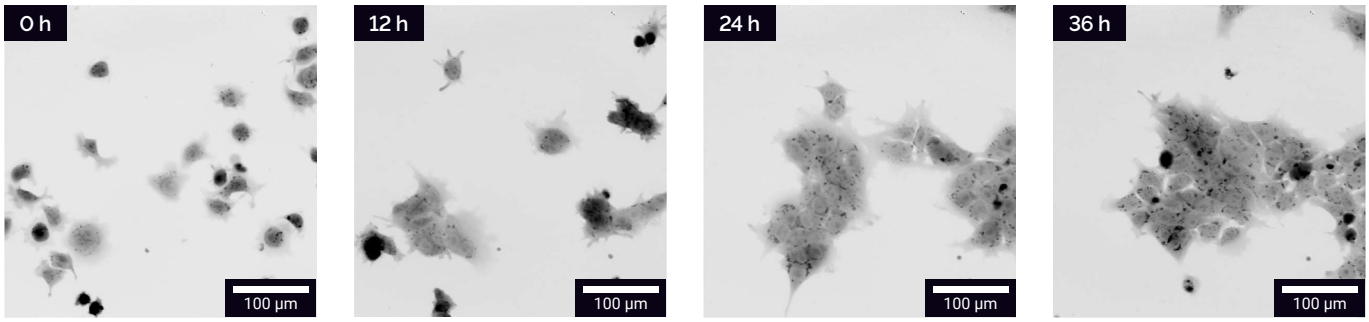


▲ Quantification of cellular uptake of doxorubicin (DOX) encapsulated nanocarriers in prostate cancer cells LNCaP. DOX fluorescence micrographs at indicated time points (top panel) were obtained from continuous quantitative phase imaging of cells treated with studied compounds (bottom panel).

For more details see: 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 8:8867, 2018.

Stem cell research: Long time-lapse, label-free imaging of cell behavior

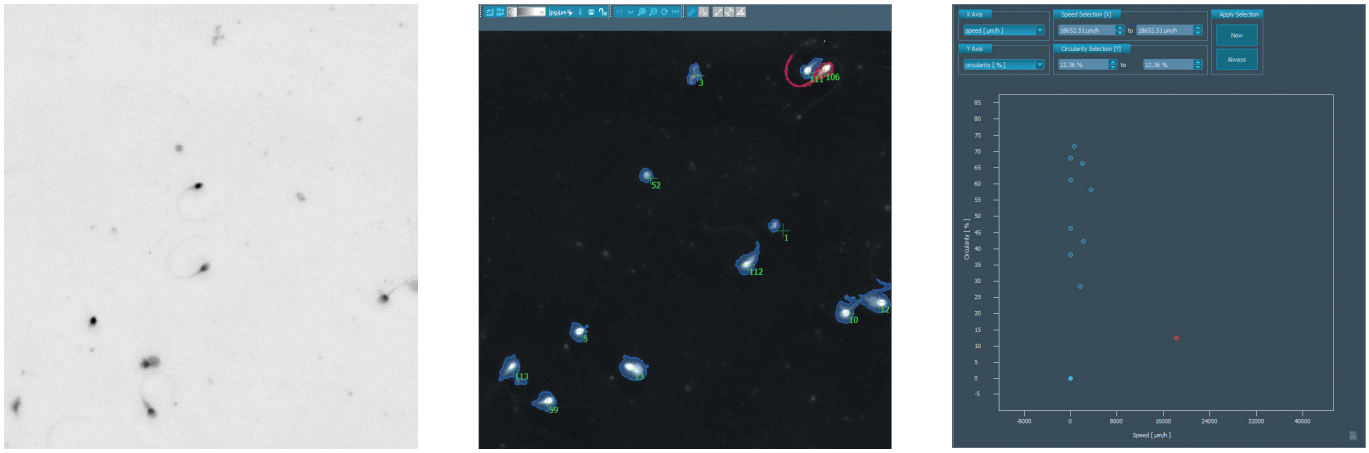
The ability of stem cells to differentiate into specialised cell types presents a number of opportunities for regenerative medicine, stem cell therapy and developmental biology. However, traditional assessments of stem cells are destructive, time consuming, and logistically intensive. Q-PHASE enables a non-invasive, label-free approach to study cell differentiation and provides a rapid, high-content characterisation of cell and tissue cultures.



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

Reproductive medicine: Motion analysis of sperm cells

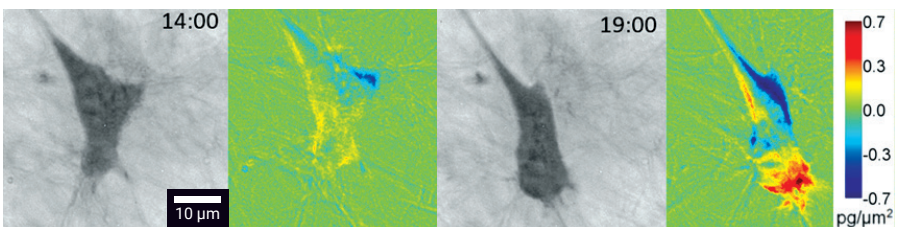
Sperm analysis, also known as a sperm count test, analyses the health and viability of human sperm. Sperm analysis measures three major factors influencing sperm health: number of sperm, shape of sperm, and movement of the sperm. However, sperm cells are normally difficult to segment and analyze from the standard microscopic images. Q-PHASE provides a fast and reliable segmentation of sperm cells, thus facilitating an assesment of the amount and quality of a man´s semen.



▲ Semen analysis by Q-PHASE system

Imaging in 3D matrix and through opaque environment: Cells in collagen matrix

Observation and analysis of cancer cell behavior in 3D environment is essential for full understanding of the mechanisms of tumor invasiveness and metastasis formation. However, such processes are not detectable by common label-free imaging methods. The coherence-gating effect, a special feature enabled by Q-PHASE, makes it possible to study dynamics of cancer cells even in scattering milieu such as the 3D collagen matrix.



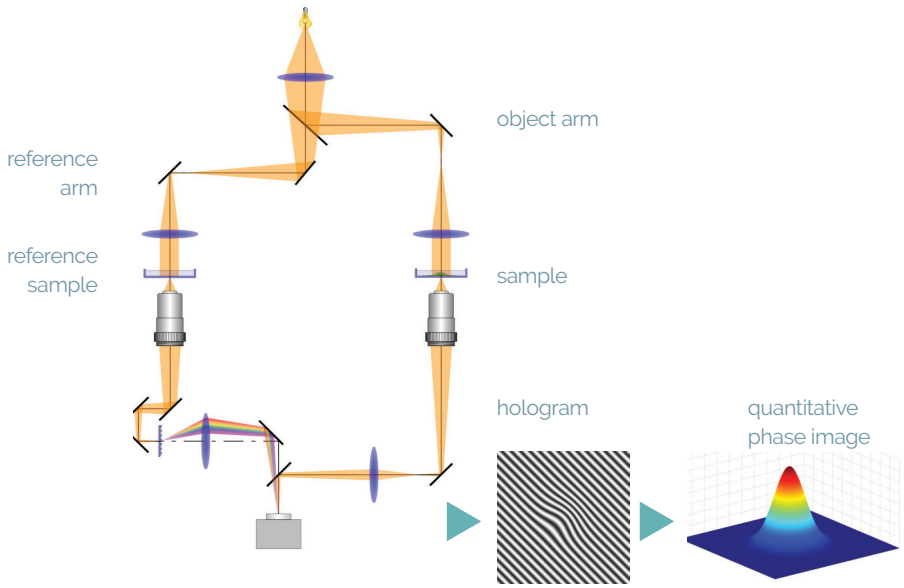
▶ Migration of mesenchymal HT1080 cell within collagen matrix. Changes of mass distribution in migrating cell were analyzed by calculating the dynamic phase differences between consequent images.

Adapted from: O. Tolde, et al.: Quantitative phase imaging unravels new insight into dynamics of mesenchymal and amoeboid cancer cell invasion, Scientific Reports 8:12020, 2018.

The Principle behind Q-PHASE

Patented Optical Setup

The Q-PHASE microscope consists of two arms, the object arm and reference arm. Both arms have similar microscope setups with a common illumination system. The sample is placed into the object arm, and the so-called reference sample (blank) is placed into the reference arm. The beams in each arm pass through the inserted sample and are combined at the image plane of the microscope. Thanks to the Q-PHASE's unique patented optical setup, the beams interfere and form a hologram even when illuminated with a halogen lamp or a LED. The hologram is then recorded by a detector and a quantitative phase image is extracted from the hologram in real time by a computer.



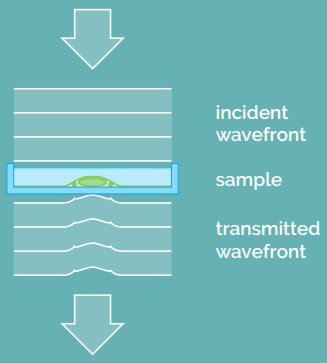
▲ Patented optical setup of Q-PHASE

Quantitative Phase Imaging

The time of propagation of light through a specific environment depends on the **refractive index** and the **distance of the optical path.** When a light wave travels through a sample with varying refractive index and/or height, its wavefront is distorted causing a change in the phase distribution of the wave. The Q-PHASE allows detecting the phase distribution in the sample plane. The process of phase detection at a sample plane is usually referred to as **quantitative phase imaging.**

Q-PHASE is based on a patented technology of coherence-controlled holographic microscopy using an incoherent light source (i.e. a halogen lamp or LED) to generate high quality QPI images without any compromises.

Sensitive mass detection by QPI



High cellular contrast
Extreme thickness sensitivity
Very low background
Quantitative data

Specifications

Microscope

Microscope configuration	transmission inverted microscope
Microscopy techniques	holography (quantitative phase imaging), epifluorescence, simulated DIC, brightfield, high-pass filtered phase
Objectives	magnification 4× to 60×
Objective turret	6-position, motorized exchange
Light source	halogen lamp
Operating wavelength	650 nm
Sample stage	motorized, 130 mm × 90 mm travel range
Focusing	motorized objective turret, 8 mm travel range
Piezo-focusing	optional, travel range 500 µm
Lateral resolution	3.3 µm with 4× NA 0.1 objective 0.57 µm with 60× NA 1.4 objective
Field of view	objective dependent, up to 1.7 mm × 1.7 mm with 4× objective
Acquisition framerate	5.5 fps at full frame (option: higher framerates possible)
Reconstructed phase image size	1200 px × 1200 px
Illumination power at sample plane	down to 0.2 µW/cm2
Phase detection sensitivity	down to 0.0035 rad (0.7 nm at Δn = 0.5) Δn - difference between refractive indexes of sample and surrounding media
Power	230 V/50 Hz (120 V/60 Hz optional), 1200 VA
Dimensions (W × L × H)	1100 mm × 950 mm × 1620 mm microscope with incubator 2515 mm × 974 mm × 1620 mm total with operator table
Weight	350 kg (including microscope table, fluorescence module and microscope incubator)
Field and aperture diaphragms	
Side port available for fluorescence module or other additional techniques	
Microscope table with anti-vibration suspension	
Control panel with multifunctional touchscreen, sample stage joystick and rotary knobs	
Microscope incubator with computer temperature setting and temperature data logging (optional)	
Incubation chamber for precise and long-term control of temperature, humidity and CO2 concentrations (optional)	

Fluorescence module (optional)

Light engines	Lumencor with 3 channels (optionally up to 5 channels)
Detectors	standard CCD 1.4 Mpix (1392 px × 1040 px) optional high-sensitivity sCMOS 5.5 Mpix (2560 px × 2160 px)
Filters	3 multichannel filter cubes, motorized channel switching

Q-PHASE users

University of North Florida & Mayo Clinic, Jacksonville, USA

- Cancer research

Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG), Dresden, Germany

- Quantitative analysis of protein droplets, mouse skull progenitors, growth & degrowth in planarian flatworms

Masaryk University Brno, Czech Republic, Faculty of Medicine, Department of Pathological Physiology

- 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 8:8867, 2018.
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