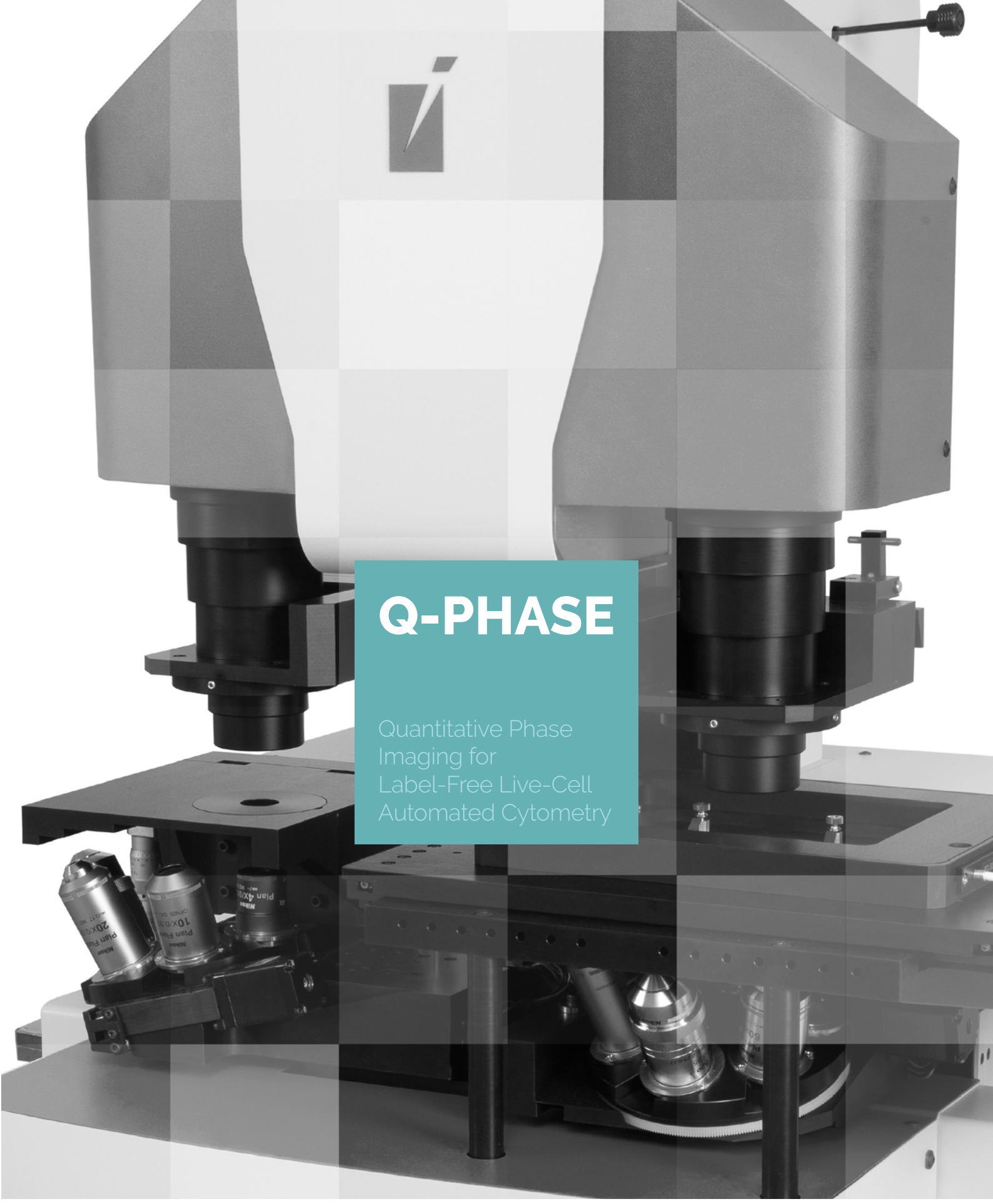


Q-PHASE

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

TESCAN

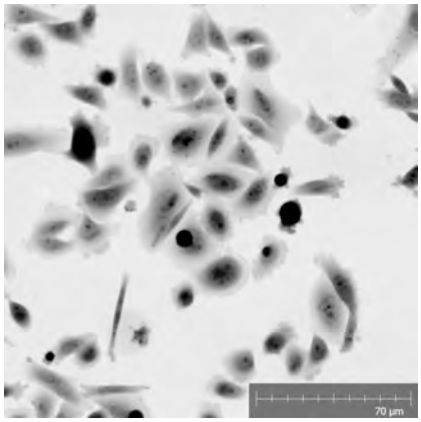
PERFORMANCE IN NANOSPACE

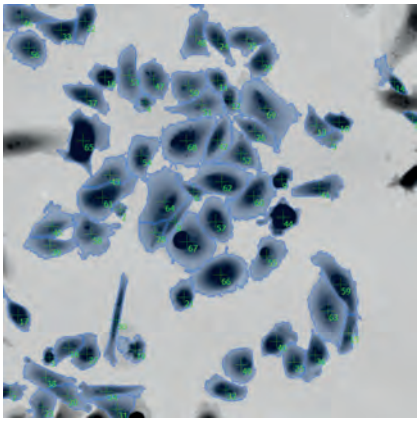
Q-PHASE holographic microscope

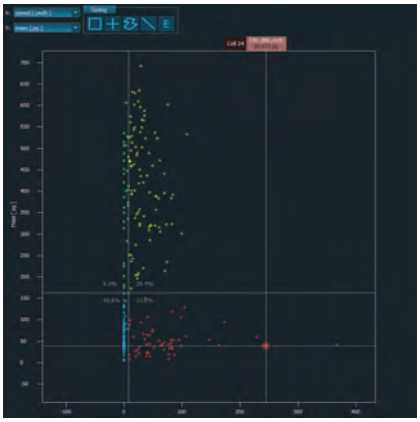
Unique holographic microscope for label-free live-cell automated cytometry

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 directly measures the optical properties (refractive index) and thickness of cells, thus providing an unparalleled view of living cells. The Q-PHASE's **unique, patented QPI concept** allows straightforward detecting 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, simulated DIC and brightfield modules. Correlative imaging between the different modes is also possible.

OBSERVE › SEGMENT › ANALYZE







Key features:

- True, label-free imaging cytometry
- 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
- Multichannel fluorescence imaging
- Comprehensive data analysis software
- All modalities can be collected simultaneously

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.

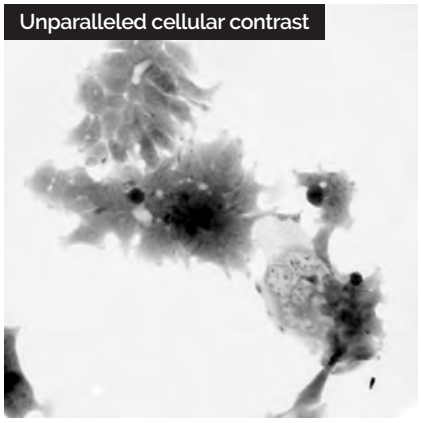
- **Cell biology**
  - Proliferation and differentation
  - Cell shape and volume
  - Motility
- **Synthetic Biology**
  - Coacervates
  - Transparent and phase objects observation
- **Cancer research**
  - Migration assay
  - Speed measurement
  - Cell-to-cell interaction
- **Host Pathogen interactions**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

OBSERVE

Cells with unmatched resolution

Q-PHASE microscope has been designed to image the cells with unmatched clarity and without any labelling. A unique patented setup of QPI, allows clearly detecting cellular boundaries and mass changes inside the cells.

Unparalleled cellular contrast

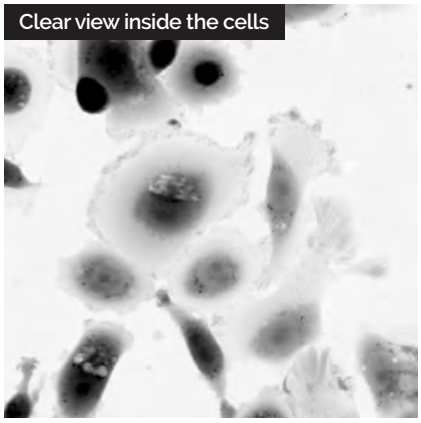


QPI directly measures optical properties and thickness of cells, thus providing an unparalleled view of living cells without using any labelling.

Transparent cell made visible

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.

Clear view inside the cells

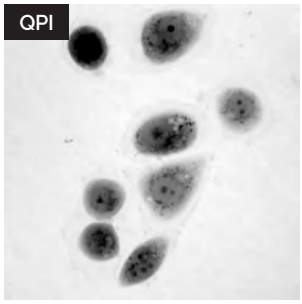


The QPI's extraordinary mass detection allows detecting changes in internal parts of the cells, such as nuclei, vacuoles, etc. without any labelling.

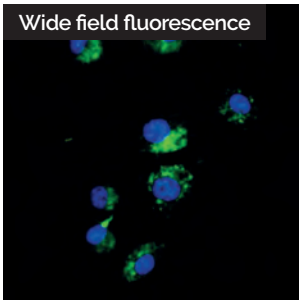
Multimodal imaging available with the Q-PHASE platform

Q-PHASE system includes fully integrated fluorescence module, simulated DIC and brightfield for fully automated multimodal imaging of the sample.

QPI

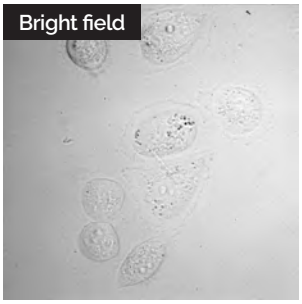


Wide field fluorescence

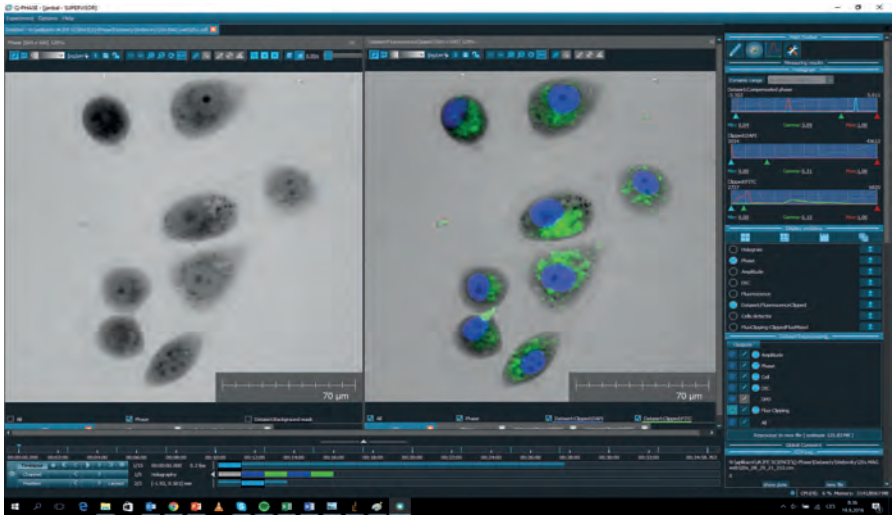


Simulated DIC

Bright field



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



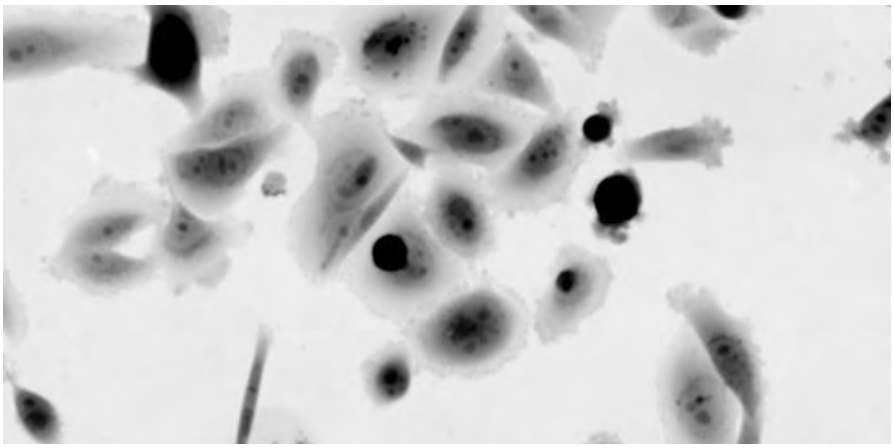
All modalities can be simultaneously collected

◄ Detection of autophagy in A2780 ovarian cancer cell line (Cyto ID – green; Hoechst 33342 – blue)

Fully automated label-free image segmentation

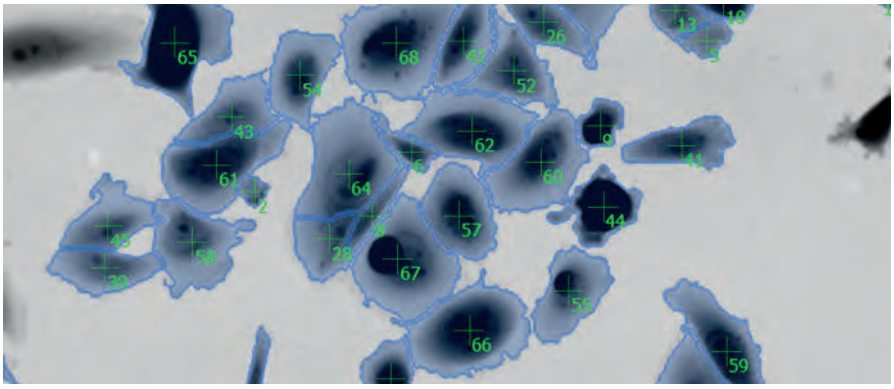
Automatically SEGMENT the data with high accuracy

High clarity QPI data allows quick automatic image segmentation based on precise detection of cellular boundaries and quantitative mass distribution of individual cells in large populations with the quality comparable to fluorescence data processing.

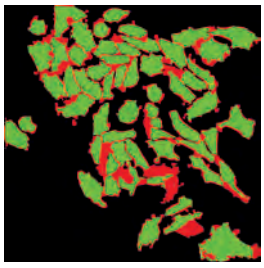
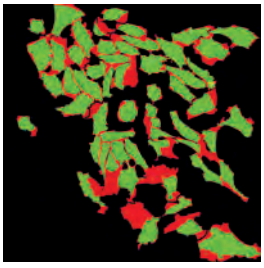
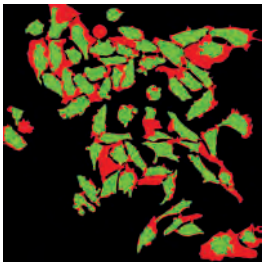
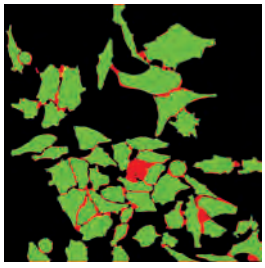


- **QPI overcomes difficulties with segmentation of bright field images**

QPI images offer the highest cell/ background contrast among other imaging techniques thus enabling the most reliable and precise segmentation



- **Precise cell detection and segmentation are essential factors for correct analysis of cell population kinetic.**

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

ANALYZE

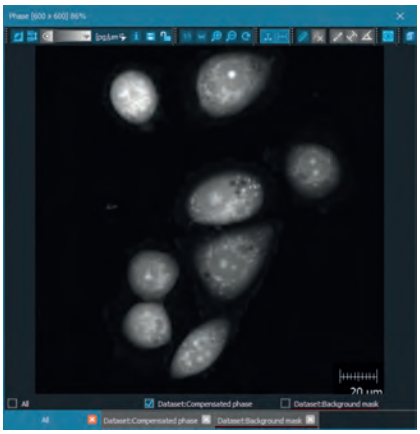
The best in class label-free image analysis software

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

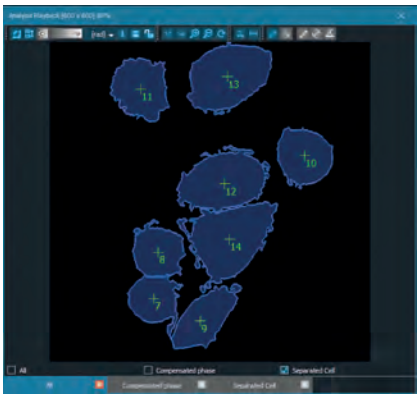
The Q-PHASE cell analyser processes segmented quantitative QPI data on-the-fly and provide a complex portfolio of tools for data visualization, subpopulation gating and multi-parameter data mining. Sample preparation is quite simple and requires minimum effort as there is no need for labelling the sample in order to resolve and distinguish the components of cell from the background. This makes it easier to keep track of cellular mass changes in individual cells as well as whole clusters of cells and their dynamics

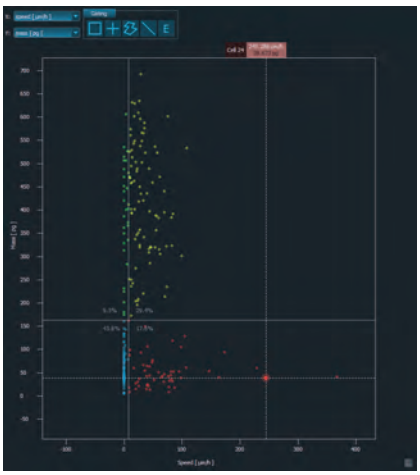
Parameters

- Cell mass
- Circularity
- Perimeter
- Confluence
- Speed
- Growth speed
- Fluorescence Average
- Fluorescence Sum.
- Motility
- Area

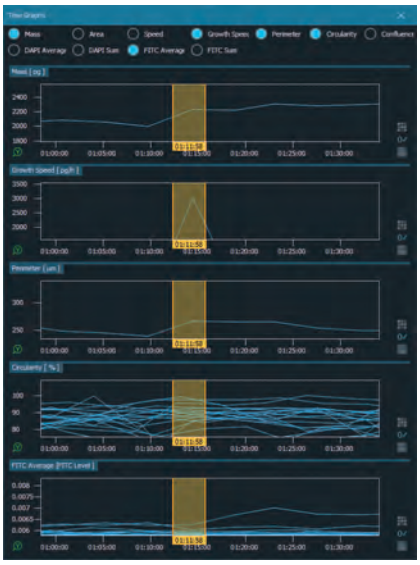


▲ Automatic/Manual segmentation parameters



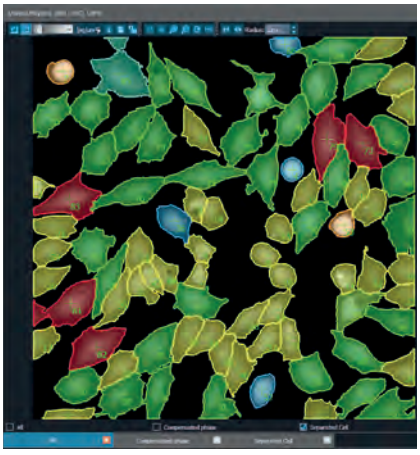


▲ Scatter plots



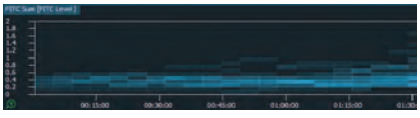
▲ Time plots

▲ Histograms



▲ Population gating

▲ Motion tracking

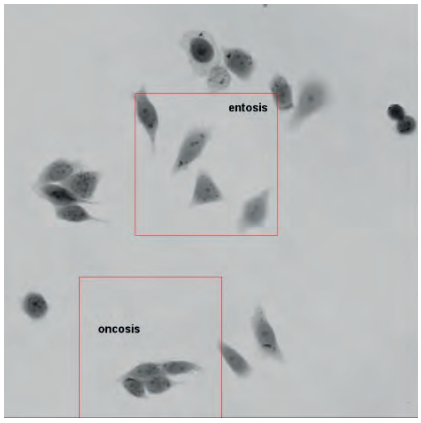


▲ Heat maps

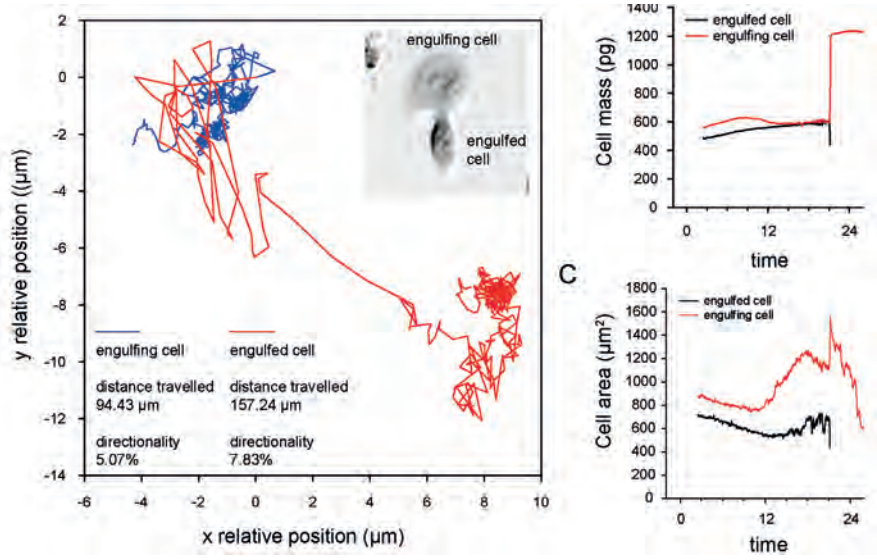
Application Examples

Cancer biology: Label free image cytometry

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, confluency, directionality, growth 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.

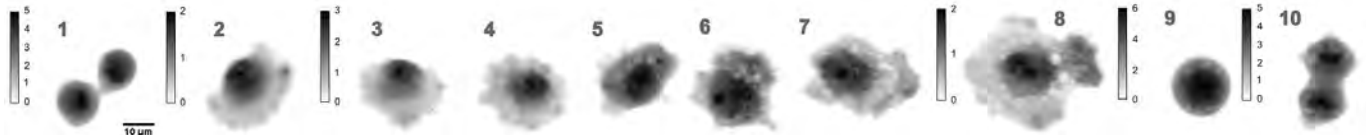


- Identification of individual cell behavior
- Detection of rare cellular events
- Clear insight into various cellular processes

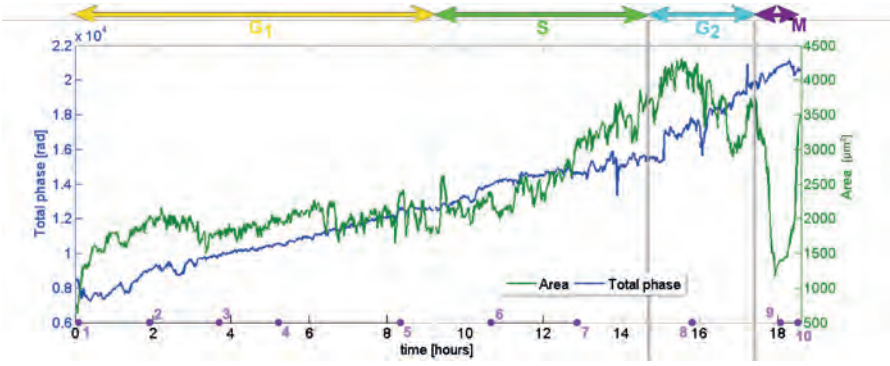


Monitoring of cell growth during the cell cycle

Quantitative measurement of cell cycle progression in individual cells over time is important in understanding drug treatment effects on cancer cells.

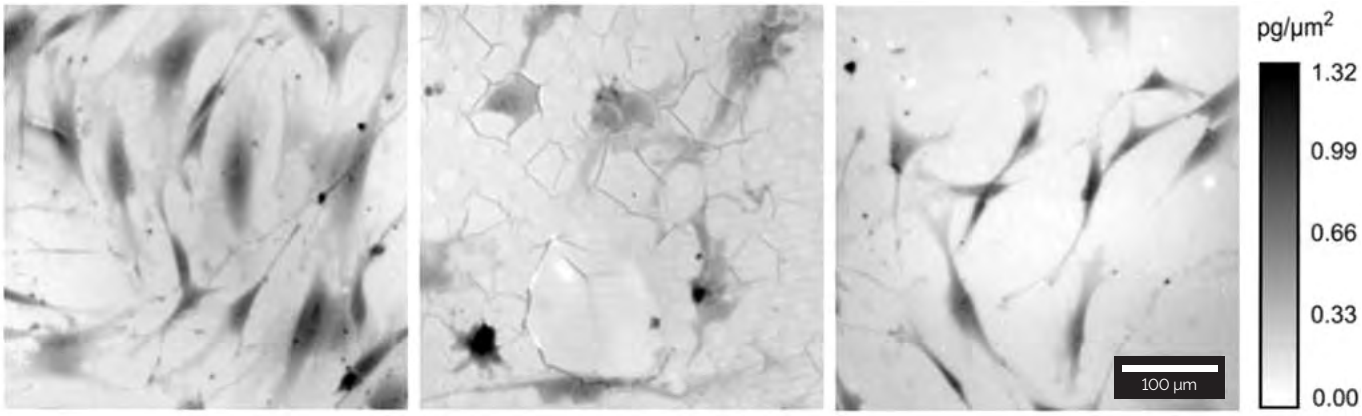


- Illustration of cell shape at marked out points in the cell life cycle
- Cell dry-mass and cell area are plotted during the cell cycle
- The value of dry-mass has been doubled between the two mitosis



Biocompatibility testing

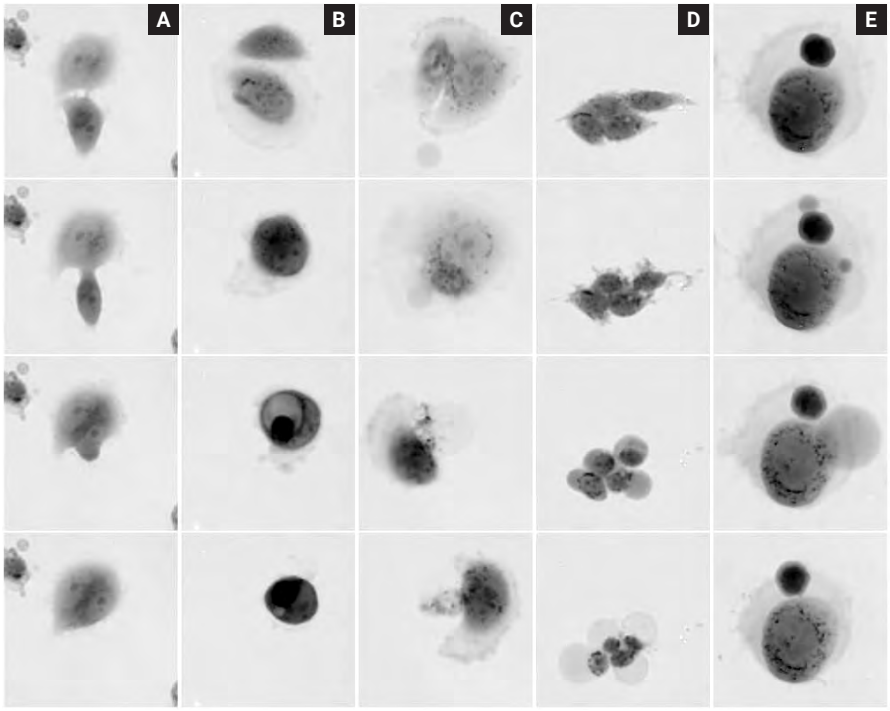
The understanding of cell–surface interactions plays an important role for biomaterials development and bioengineering. Normal human dermal fibroblasts are chosen for monitoring of biological response to the properties of amine layers. The coherence-controlled holographic microscopy (CCHM) enabled by 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 plasma-treated surfaces with different properties can be studied and evaluated.



▲ **Quantitative phase images obtained by CCHM.** LF cells on the CPA40 (left), CPA42 (middle) and control (right) sample 4 days after seeding. Scale bar and calibration bar apply to the three images. Taken from: Strbkova L, Manakhov A, Zajickova L, Stoica A, Vesely P, Chmelik R. The adhesion of normal human dermal fibroblasts to the cyclopropylamine plasma polymers studied by holographic microscopy. Surface & Coatings Technology 2016; **295**:70-77.

Cancer Cell Biology

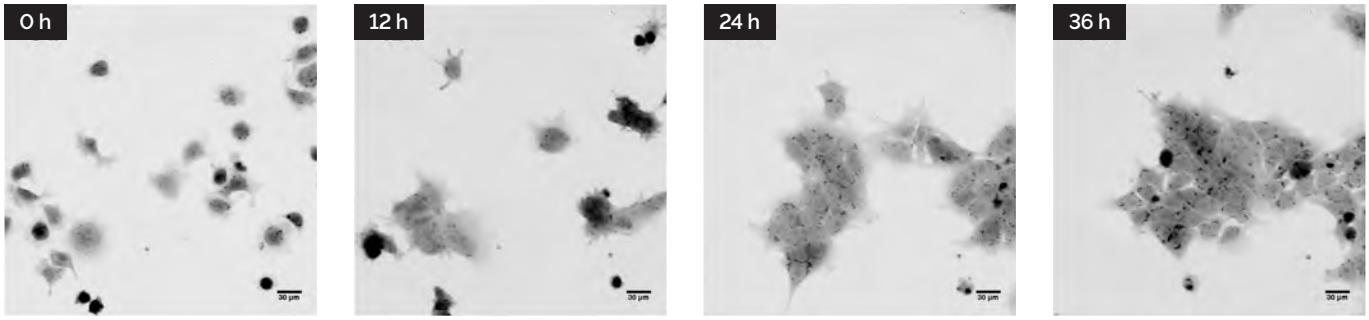
QPI allows detailed assessment of cell attributes due to the extremely high sensitivity in detecting even the smallest changes in mass density. This in turn, allows very good segmentation of individual cells and further in-depth analysis of many cellular parameters, such as mass changes, confluency, directionality, growth 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.



▶ QPI time-lapse of cell interactions.
- (A) Time-lapse imaging of entosis.
- (B)Time-lapse imaging of cell fusion with cannibalism (digestion of engulfed cell).
- (C) Time-lapse imaging of cannibalism without fusion.
- (D) Time-lapse imaging of oncosis.
- (E) Time-lapse imaging of reverse oncosis.

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

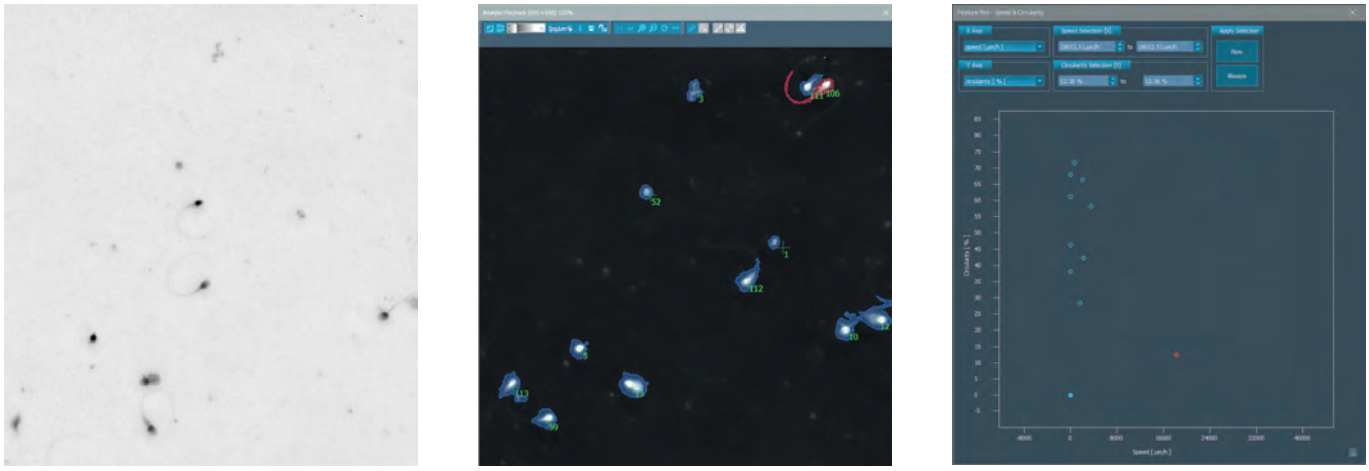
The ability of stem cells to differentiate into specialised cell types presents a number of opportunities for regenerative medicine, stem cell therapy and developmental biology. However, traditional assessments of stem cells are destructive, time consuming, and logistically intensive. Q-PHASE enables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



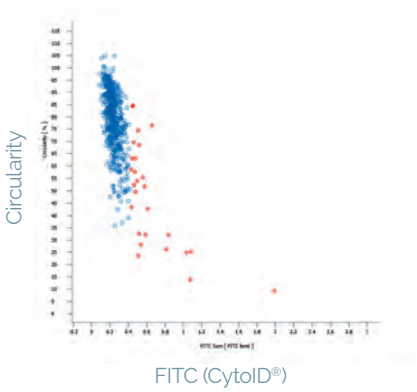
▲ Samples provided by Josef Jaroš, Faculty of Medicine, Masaryk University, Brno

Reproductive medicine: Motion analysis of sperm cells

Sperm analysis, also known as a sperm count test, analyses the health and viability of human sperm. Semen is a fluid containing sperm, sugar and protein substances that nourish the sperm. A semen analysis measures three major factors influencing sperm health: number of sperm, shape of sperm, and movement of the sperm. Sperm cells are difficult to segment from the images. Q-PHASE provides a fast and reliable segmentation of sperm cells.



CytoID, Hoechst 33342 labeling: Autophagic cells (CytoID positive cells) are less circular then other cells (presence of acidic autophagosomes).



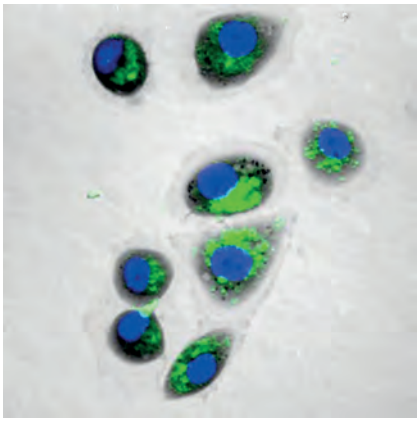
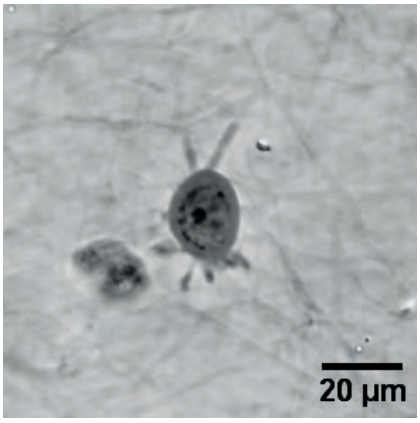


Image in 3D matrix and through opaque environment: Cells in collagen matrix

- Q-PHASE can observe through lightly scattering media
- Basic biological test of cancer cell invasiveness
- Recorded mechanism of cell motion, not detectable by common label-free methods
- 3D gel mimics in vivo situation and thus makes study of dynamics of cancer cell reactions to the surroundings more realistic

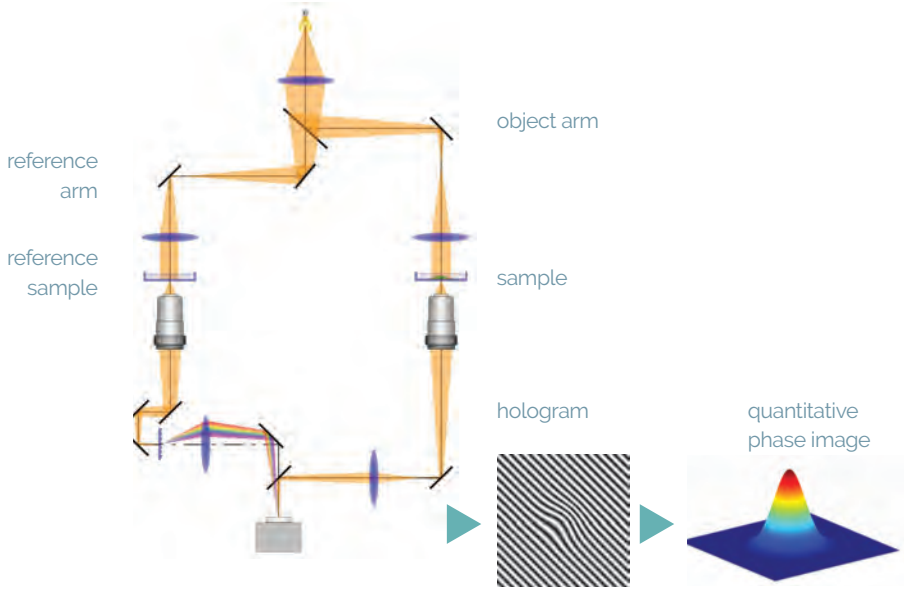


▶ QPI, human sarcoma cells (MCF7), objective 20x/0.4, interval of acquisition 20 s, mesenchymal motion

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.



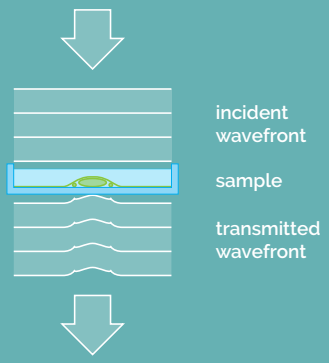
▶ Patented optical setup of Q-PHASE

Coherence Controlled Holographic 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 (darker pixel => cellular)

Specifications

Microscope

Microscope configuration	transmission inverted microscope
Microscopy techniques	holography (quantitative phase imaging), epifluorescence, simulated DIC, brightfield
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), 2300 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

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

- J. Balvan, et al., "Multimodal holographic microscopy: Distinction between apoptosis and oncosis," PLoS ONE 10, e0121674 (2015).

Brno University of Technology, Experimental Biophotonics Group

- H. Janeckova, P. Vesely, and R. Chmelik, "Proving tumour cells by acute nutritional/energy deprivation as a survival threat: a task for microscopy," Anticancer Research 29, 2339-2345 (2009).
- J. Collakova et al.: Coherence-controlled holographic microscopy enabled recognition of necrosis as the mechanism of cancer cells death after exposure to cytopathic turbis emulsion, J Biomed Opt 20 (11), 2015.

Institute of Molecular Genetics AS CR, Prague, Czech Republic, Laboratory of Light Microscopy and Cytometry

- Osmotic changes in cells, cell reaction to treatment, cells in 3D environment

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