Q-PHASE

MULTIMODAL HOLOGRAPHIC MICROSCOPE

Quantitative phase imaging

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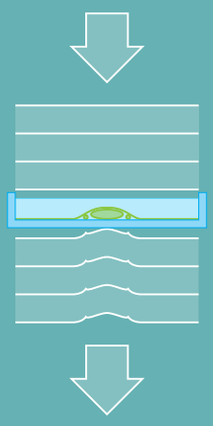
Introduction

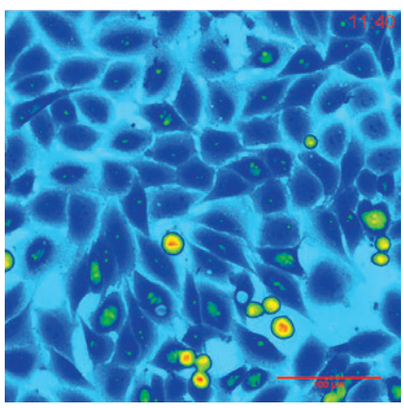
TESCAN proudly introduces the Q-PHASE, a multimodal holographic microscope (MHM). With this instrument TESCAN expands into the field of advanced light microscopy. The Q-PHASE is a unique instrument for quantitative phase imaging (QPI) based on patented technology of Coherence-controlled holographic microscopy. [1,2] This technology uses incoherent light sources (halogen lamp, LED) providing QPI with the highest quality, without any compromises and it is the only QPI technique enabling imaging of samples in scattering media. The Q-PHASE is purposely designed to observe living cells *in vitro*. It is based on a robust inverted transmission microscope platform. The whole system is situated in a microscope incubator. The full motorization fulfills even the highest demands regarding experiment automation. Furthermore, this system includes multiple imaging modes with fully integrated **Fluorescence Module, simulated DIC and brightfield imaging options.** All these features make of Q-PHASE a valuable research tool for biological and biotechnical applications such as testing reactions of cells to a specific treatment - even with scattering **non-transparent substances,** monitoring cell's life cycle including mitosis, distinguishing between different forms of cell deaths, analyzing cell growth, motility or morphology changes, imaging cells in extracellular matrices. [3,4]

Principles of quantitative phase imaging

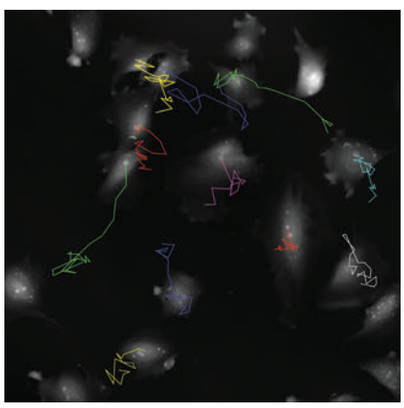
The time of propagation of light in a specific environment depends on the refractive index as well as the distance of the optical path. Therefore, when a light wave travels through a sample with varying refractive index and/or height, its wavefront is distorted and a change in the phase distribution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



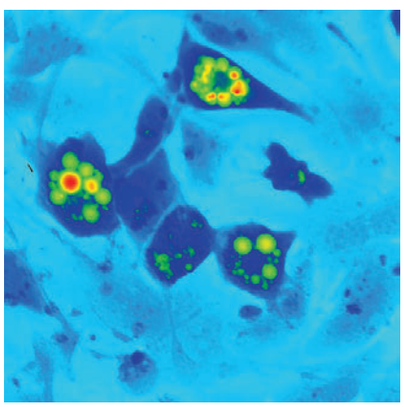
Quantitative phase image can provide information on sample morphology, topography or cell dry-mass distribution. [5,6] Cell dry mass is quantified in pg/μm^2 and can be calculated directly from phase values detected in each pixel. Quantitative phase imaging provides very simple and sensitive way for monitoring of cell reactions to treatment and analyses of movement, growth, area, shape and many other parameters. Various color LUTs are often used for representation of phase images to easily distinguish different phase values.



▲ QPI of non-metastatic fibroblast-like LW13K2 (K2) cells



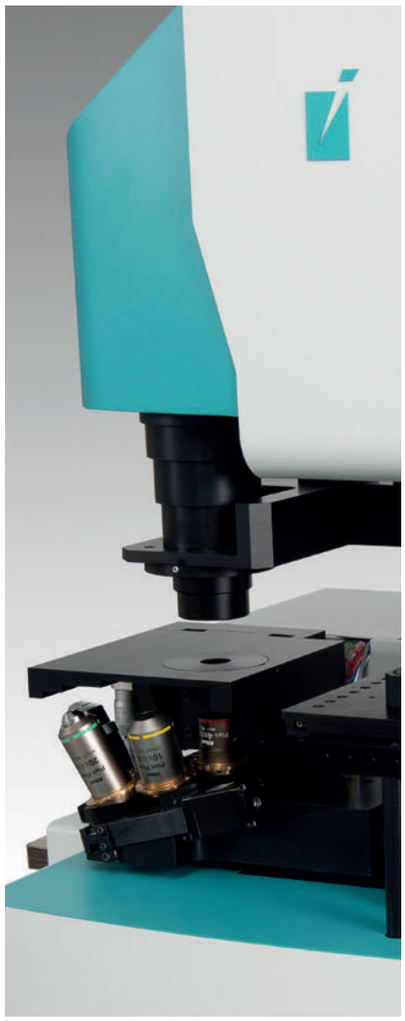
▲ QPI of rat sarcoma cells, cell tracking



▲ QPI of human adipocytes

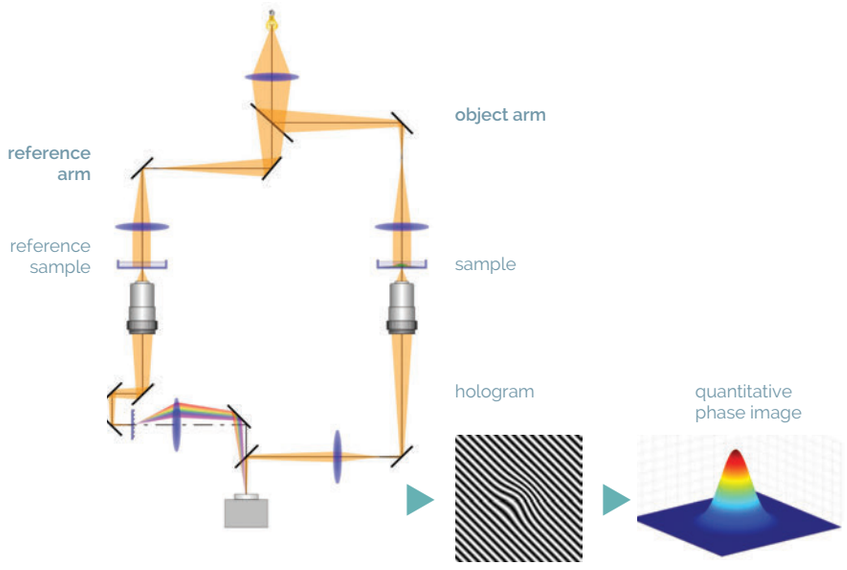
Key features and advantages

- **No image artifacts such as halo effect** (as opposed to techniques based on Zernike phase contrast illumination)
- **Enables very precise detection** of cell boundaries
- **Strong suppression of coherent noise** (speckles) & parasitic interferences (as opposed to laser-based approaches)
- **Label-free** – no staining is needed, simple sample preparation, observation of live cells in their native environment, no photobleaching problems
- **Low phototoxicity** – low light power density (10^7× lower than fluorescence microscopy) allows long-term observations (for days)
- **Coherence-gating effect** – Q-PHASE special feature enabling to observe samples even in scattering media (phospholipid emulsions, extracellular matrices, etc.)
- **Multimodality** – fully integrated fluorescence module, simulated DIC and brightfield which enables automatic multimodal imaging of the sample
- **High-quality QPI** – unique Q-PHASE's optical setup allows using incoherent illumination which provides extraordinary imaging quality without any compromises
- **Lateral resolution of conventional microscopes** (up to 2× better when compared to common laser-based approaches or pinhole spatial filtering based techniques)
- **Fast acquisition** – the use of off-axis holographic approach makes Q-PHASE a single-shot instrument, thus enabling imaging of very fast cell dynamics
- **Full motorization** – focusing, sample stage, objective exchange, fluorescence filters
- **Automated multidimensional acquisition** – time-lapse, channel, position, Z-stack
- **Simple image segmentation and processing** – comparable to fluorescence data processing
- **Quantitative** – phase values can be recalculated e.g. to cell dry-mass density (pg/μm²) or direct topography with nanometer sensitivity (usually non-biological samples with homogeneous refractive index distribution)
- **High phase detection sensitivity** – enables to detect even the smallest changes in axial direction, very sensitive detection of morphology or position changes



Patented optical setup

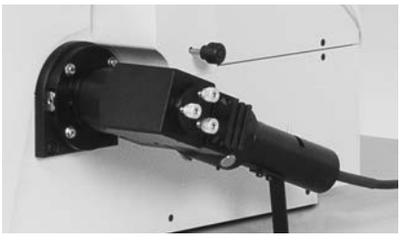
The Q-PHASE microscope consists of two arms, object arm and reference arm. The arms have similar microscope setups with a common illumination system. The sample is placed into the object arm, and the so-called reference sample (blank) is placed into the reference arm. The beams in each arm pass through 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

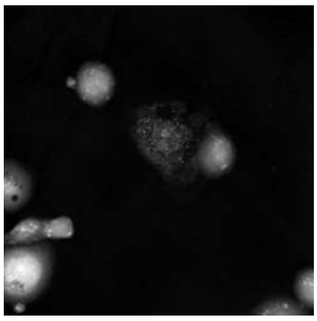
Fluorescence module

The Q-PHASE can combine holographic microscopy with the fluorescence microscopy. This powerful combination provides the possibility to verify structures or processes observed in QPI with fluorescence microscopy in the same field of view using a single instrument. For example, morphological and position changes prior to cell death can be observed in QPI with following fluorescence verification of cell death types (see images below) [4]. This approach greatly reduces the phototoxicity and photobleaching problems of fluorescence imaging and it allows long-term observations. The focus plane in both methods is located at the same position. This allows easy and fast switching between the two imaging methods at the same conditions and time points. Multiple fluorescence channels are possible with motorized channel exchange for automated multidimensional measurements. The illumination can be implemented by using liquid light guide coupled solid state light sources or a xenon arc lamp. Multidimensional image acquisition combining holography and fluorescence is fully integrated in the Q-PHASE's software. A fluorescence module is attached to the side port of the Q-PHASE, which can alternatively be used for other imaging techniques.

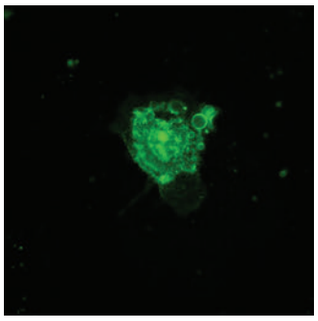


▲ Fluorescence module attached to the side port

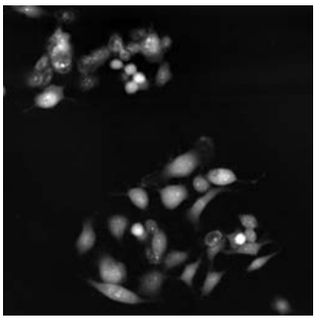
Quantitative phase image



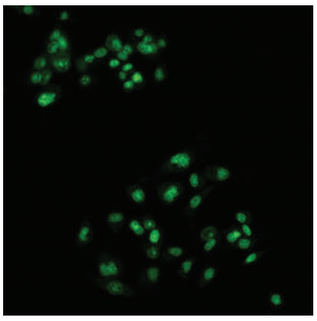
Fluorescence image (Annexin V)



Quantitative phase image



Fluorescence image (SYTO 16)

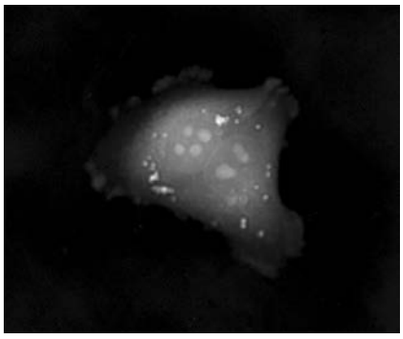


▲ Multimodal imaging of human prostate cancer cells

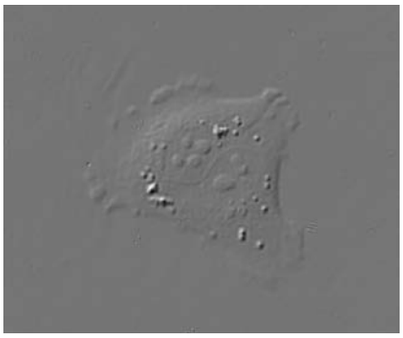
Intrinsic imaging modes

Complementary image contrast can be obtained just by numerical processing of the acquired phase images. In this way simulated DIC images can be produced with adjustable shear and displayed in real time. Another possibility is the brightfield imaging which can be simply achieved by closing the reference arm of the microscope. In summary, the Q-PHASE offers multiple imaging modes widely used in biological research such as fluorescence or DIC integrated in a single instrument and supported by the Q-PHASE's software allowing fully automated multimodal imaging.

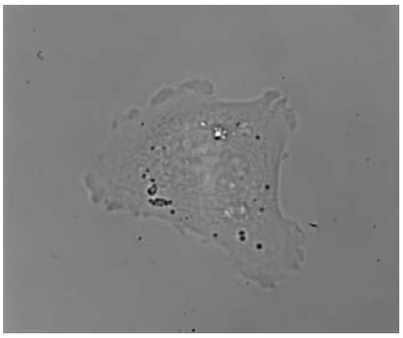
Quantitative phase image



Simulated DIC



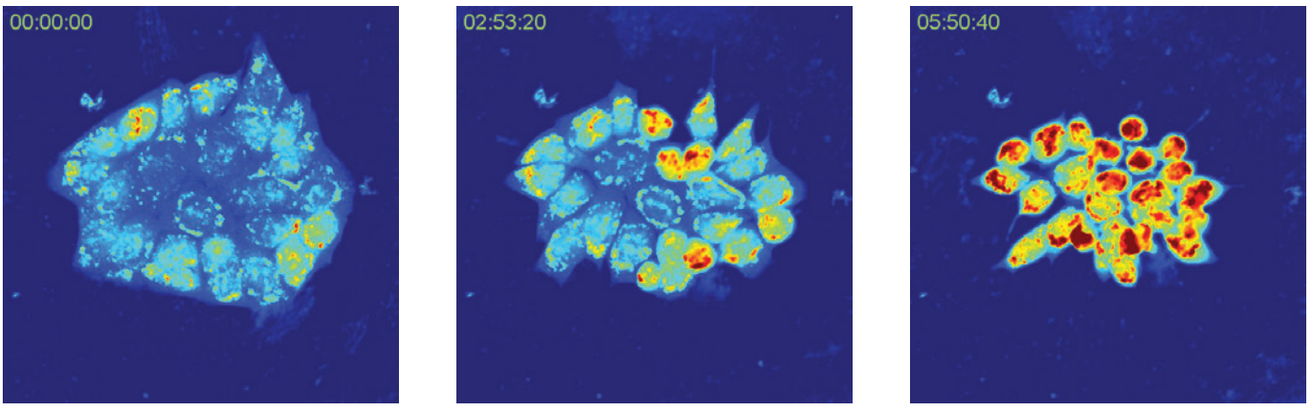
Brightfield



▲ Multimodal imaging of human malignant melanoma cells

Imaging in scattering media

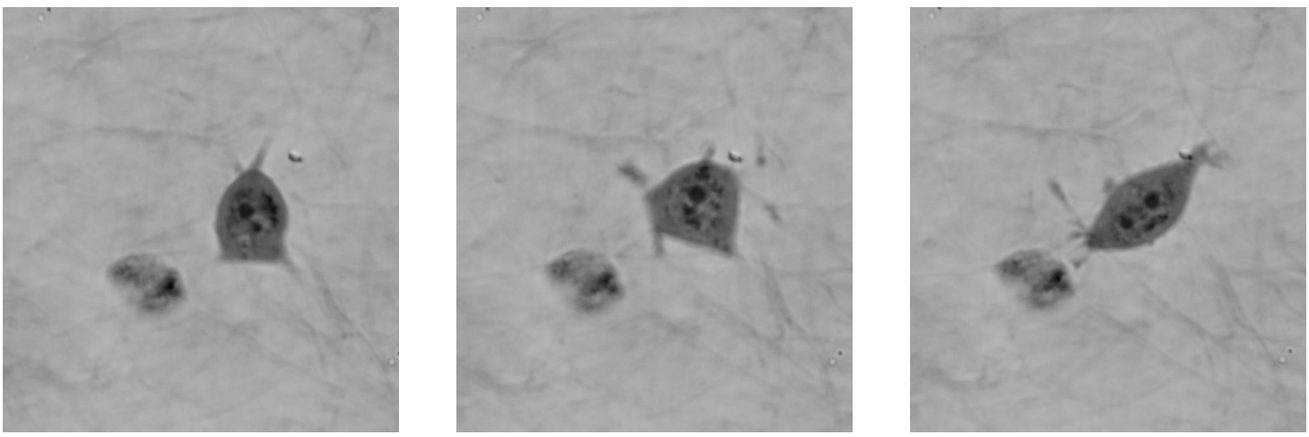
A special feature of Q-PHASE is the coherence-gating, a well-known effect in optical coherence tomography which enables observations of samples even in scattering media. This effect is induced by using incoherent light in the unique patented setup of Q-PHASE. Its transmitted-light configuration enables to effectively suppress the light which was scattered by the environment in defocused planes and to only use unscattered light for imaging. In this way, cells can be observed even in moderately scattering non- transparent substances such as an active phospholipid emulsion.



▲ QPI of reaction of human colorectal cancer cells to treatment by 0.15% active phospholipid emulsion (scattering medium)

Imaging in extracellular matrices

The coherence-gating effect can also be beneficial when imaging cells in extracellular matrices such as collagen gel. Extracellular matrices mimic *in vivo* situation making the study of the cell's dynamic reactions to its surroundings more realistic. Usually it is used as a biological test for cancer cell invasivity and ability to metastasis. The Q-PHASE microscope enables to record mechanism of cell motion and interactions between extracellular matrix fibers and cells with high contrast and without any additional staining.



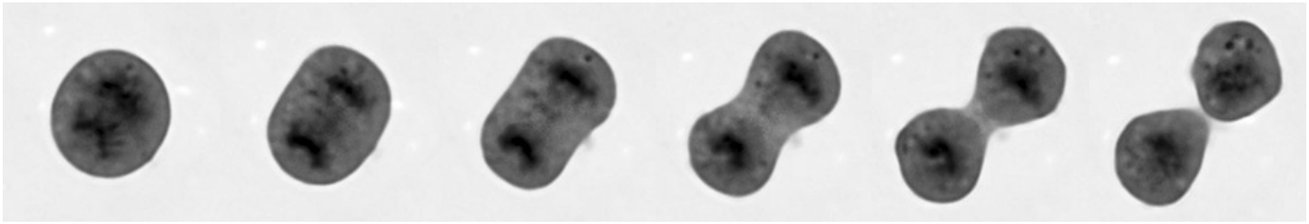
▲ QPI of human sarcoma cell motion mechanism in collagen gel (inverted LUT)

Applications

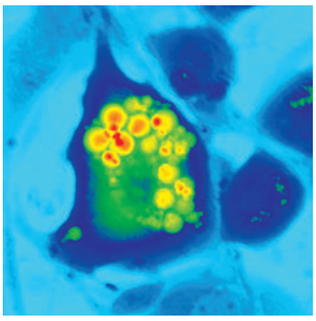
Many different types of samples can be observed including adherent cells in monolayers, thin tissue sections or plant samples. The sample preparation is quite straightforward; simply requiring to pour the cell suspension into the observing chamber with no further procedures needed. The cells can be also seeded into the perfusion chamber and perfusion system and different treatments can be applied. Cell reactions to the treatment can then be observed online.

Application examples

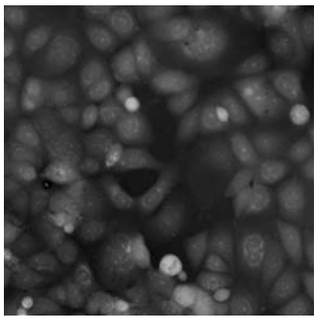
- Cell life cycle, cell proliferation, cell differentiation, cell viability, counting
- Cell dry mass evaluation, cell growth, changes in cell dry mass distributions
- Cell morphology changes, intracellular processes
- Cell motility, tracking
- Cell interactions, cell co-cultures
- Fast cellular processes
- Testing reactions of cells to a specific treatment, cytotoxicity
- Imaging in scattering non-transparent media (e.g. phospholipid emulsions), imaging in 3D environments (e.g. collagen extracellular matrix)
- Multimodal imaging (QPI automatically correlated with fluorescence, DIC or brightfield)



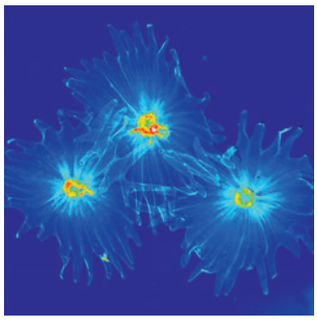
▲ QPI of rat sarcoma cell mitosis (inverted LUT, contrast enhanced by adaptive contrast control method)



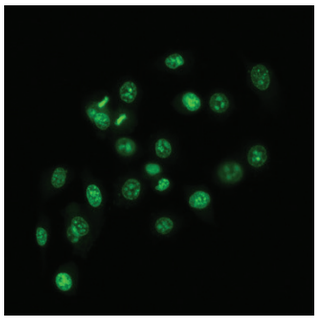
▲ QPI of human adipocytes



▲ QPI of human breast cancer cells



▲ QPI of olive leaf trichomes



▲ Fluorescence image of human prostate cancer cells (SYTO 16)

Software

The Q-PHASE's software is an integral part of this microscope. It runs on the 64-bit Windows™ edition. It is divided into two modes, Live and Data-set, which provide all the necessary functionality for experimental measurements, image processing and analysis. Simple graphical interface with tabs allows easy orientation. Exporting images to standard formats allowing to process and analyze the images with third party software. The implemented GPU acceleration gives the highest performance.

- Full microscope control (focusing, sample stage, objective exchange, fluorescence channels, light-source, shutters, camera settings)
- Multidimensional image acquisition (time-lapse, channel, position, Z-stack)
- GPU accelerated real-time holographic image reconstruction
- Multidimensional dataset viewer
- Image processing and analysis
- Microscope control with control panel
- User management
- Microscope alignment wizard with automatic procedures
- Microscope incubator control

▲ Control panel

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, multiple travel ranges available
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 950 µm × 950 µm with 4× objective
Acquisition framerate	5.5 fps at full frame (option: higher framerates possible)
Reconstructed phase image size	600 px × 600 px
Illumination power at sample plane	down to 0.2 µW/cm^2
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, epi-fluorescence attachment 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

Incubation chamber for precise and long-term control of temperature, humidity and CO_2 concentrations.

Fluorescence module (optional)

Light engines	Lumencor Aura, Lumencor Spectra, Sutter Lambda XL
Detectors	standard CCD 1.4 Mpix (1392 px × 1040 px) optional high-sensitivity sCMOS 5.5 Mpix (2560 px × 2160 px)
Filters	multichannel filter cube, motorized channel switching

References

[1] US patent No. 8526003.

[2] T. Slabý et al.: Off-axis setup taking full advantage of incoherent illumination in coherence-controlled holographic microscope. Optics Express 21, 2013, 14747.

[3] H. Janeckova et al.: Proving tumour cells by acute nutritional/energy deprivation as a survival threat: a task for microscopy. Anticancer Research 29, 2009, 2339–2345.

[4] J. Balvan et al.: Multimodal holographic microscopy: distinction between apoptosis and oncosis. PLOS ONE, 2015 (submitted).

[5] R. Barer: Interference microscopy and mass determination. Nature 169, 1952, 366-367.

[6] H. Davies, M. Wilkins: Interference microscopy and mass determination. Nature 169, 1952, 541.

WATCH Q-PHASE VIDEOS ON YouTube

Reaction of cells to treatment

Cell in collagen gel

Cell proliferation

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