

# Quantitative Phase Imaging

## (Q-PHASE Multimodal Holographic Microscope by Telight)

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Light Microscopy

IMG CAS, Prague

# Motivation

**Imaging cells ... without any labelling**

**... without image artifacts**

**... for long time**

# Invention of coherence-controlled holographic microscope

**Czech Head (Česká hlava) – Invention prize 2016**

**Prof. RNDr. Radim Chmelík, Ph.D.**

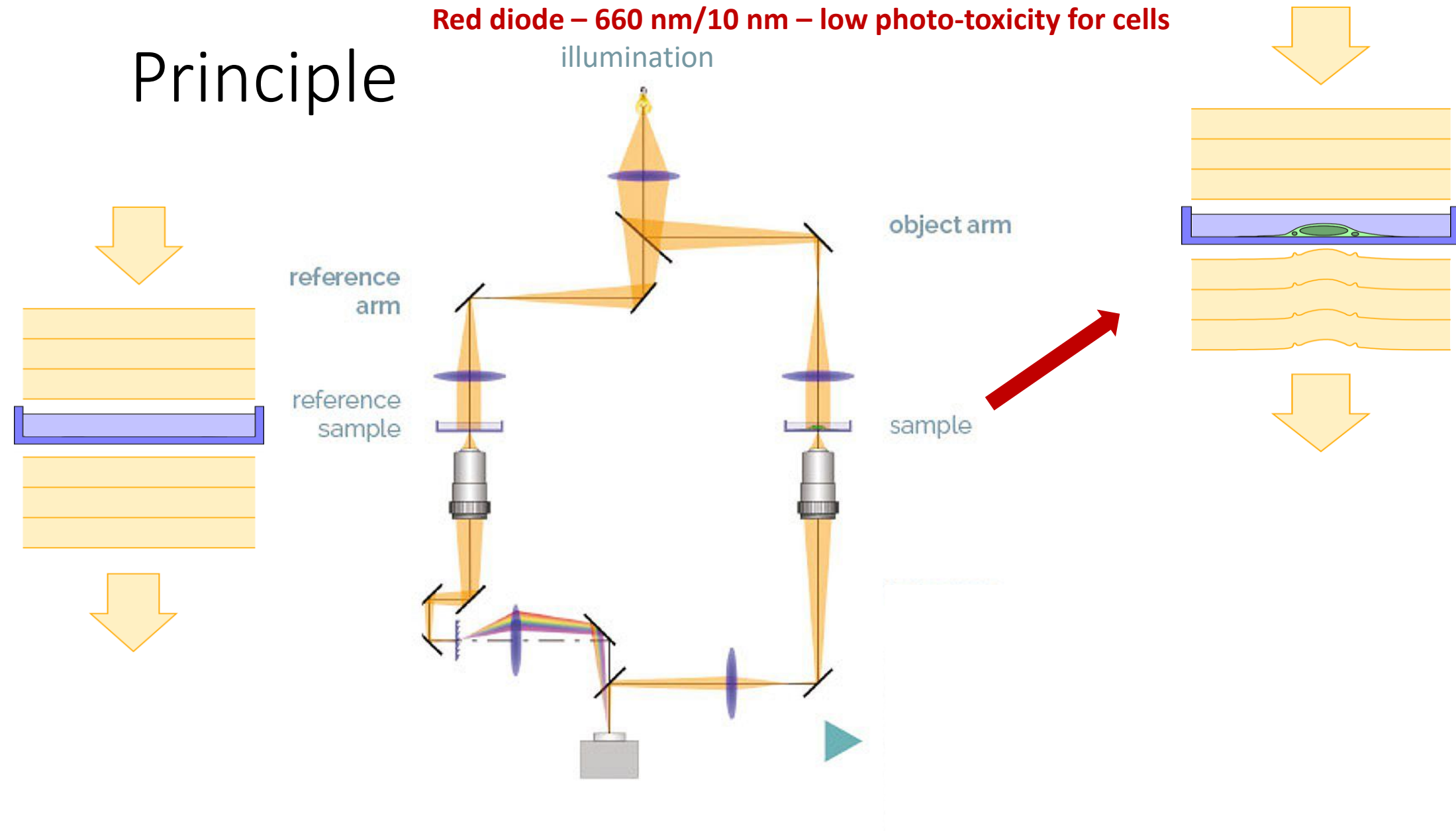
**CEITEC Brno University of Technology**

Patented in the Czech Rep, EU, USA, Japan, China  
and the countries covered by the Eurasian Patent Office.



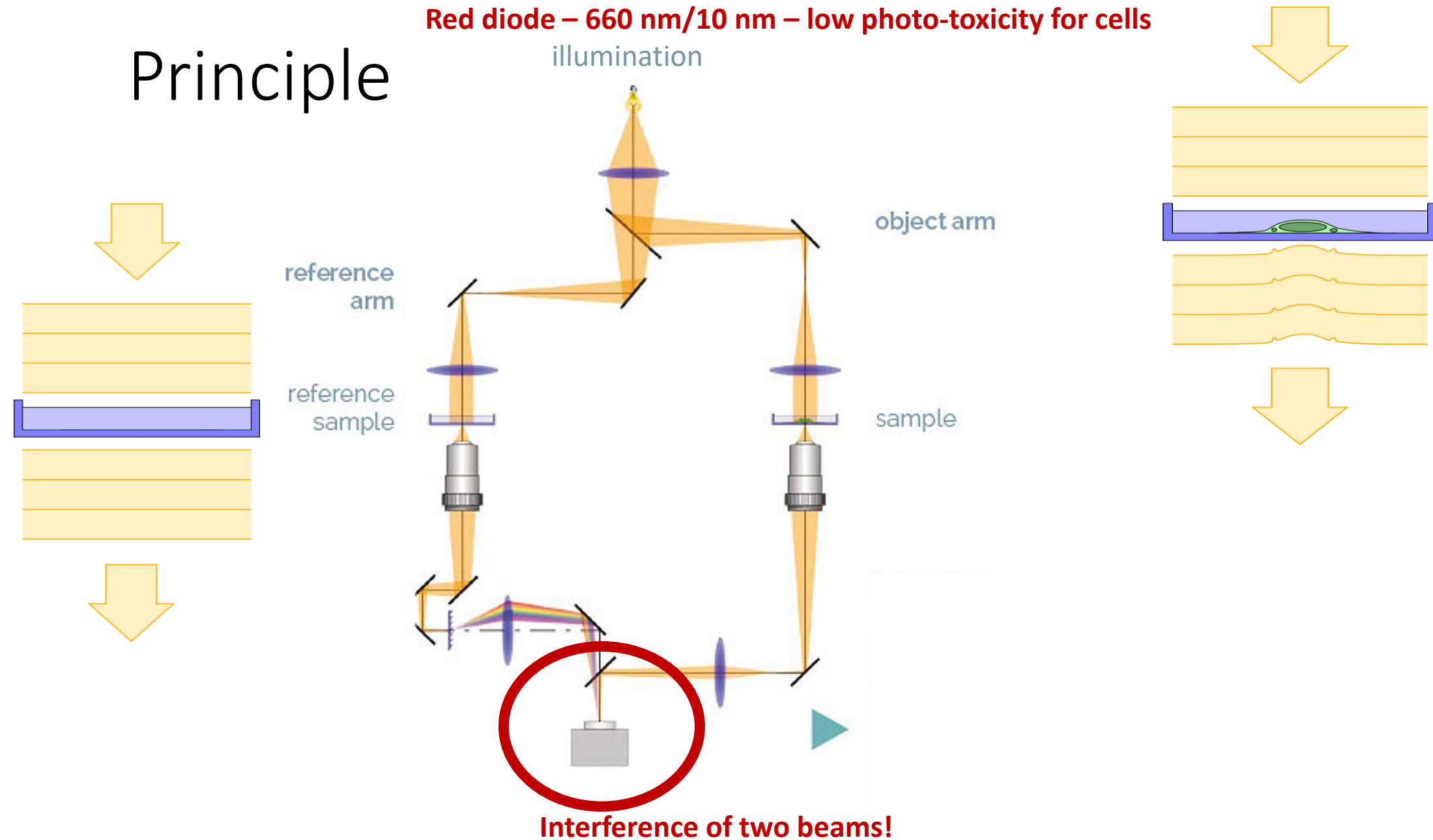
# Principle

Red diode – 660 nm/10 nm – low photo-toxicity for cells  
illumination



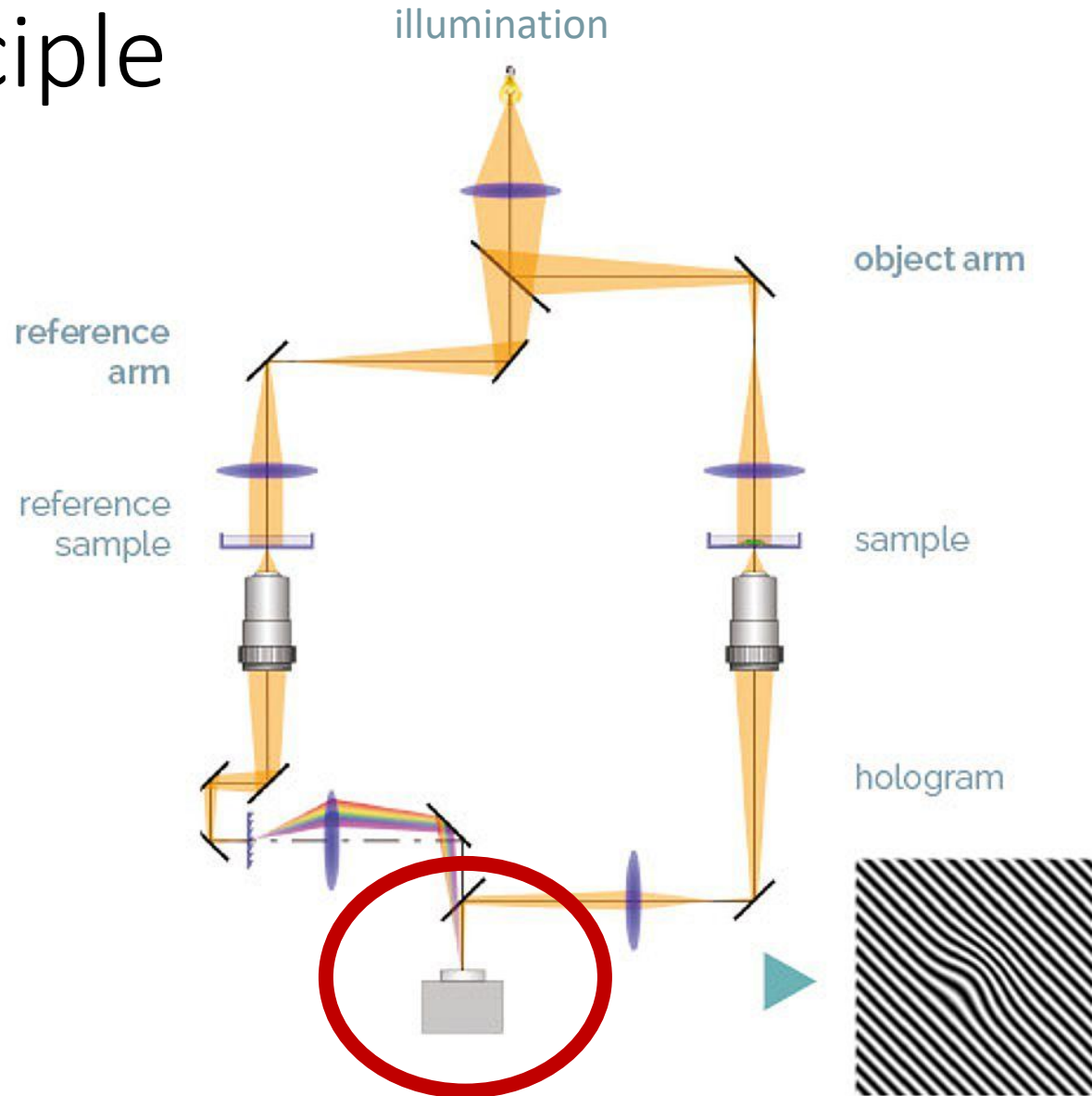
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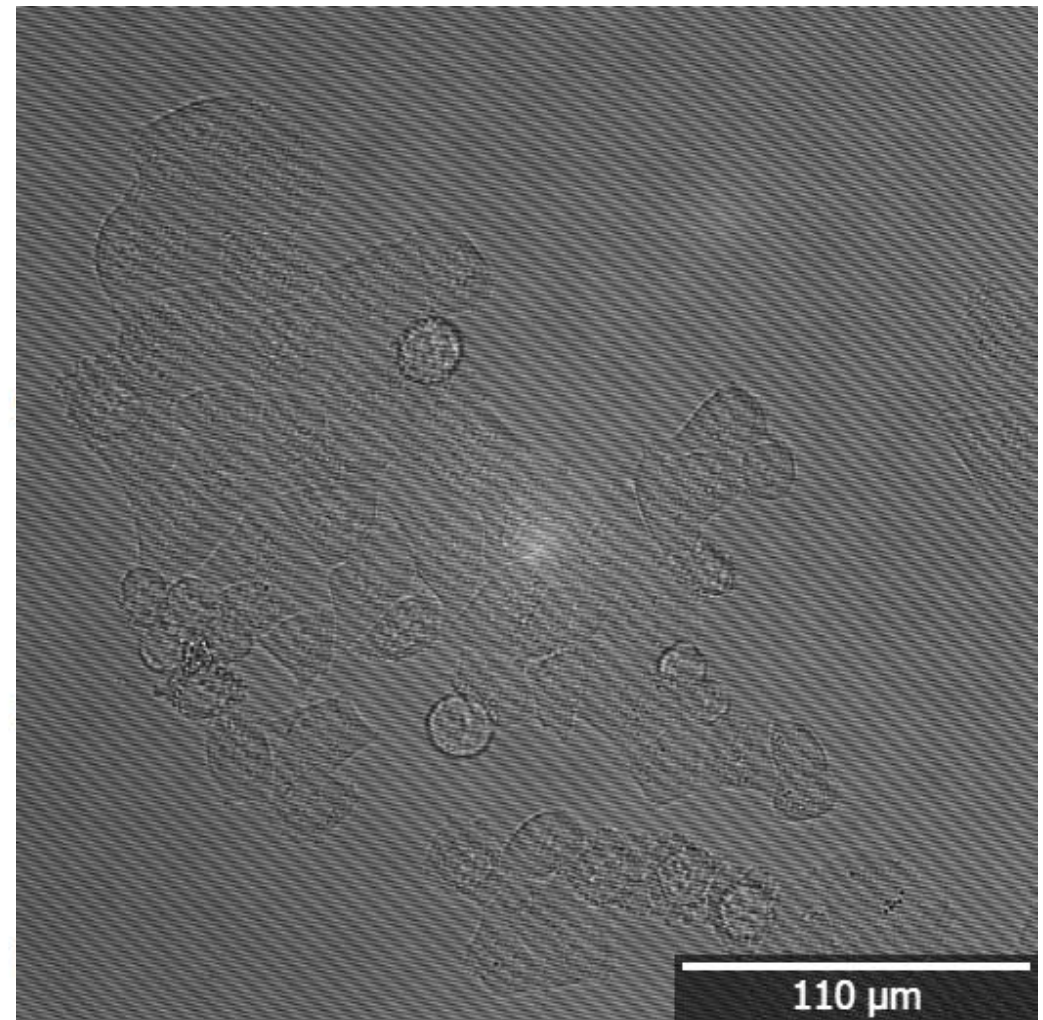
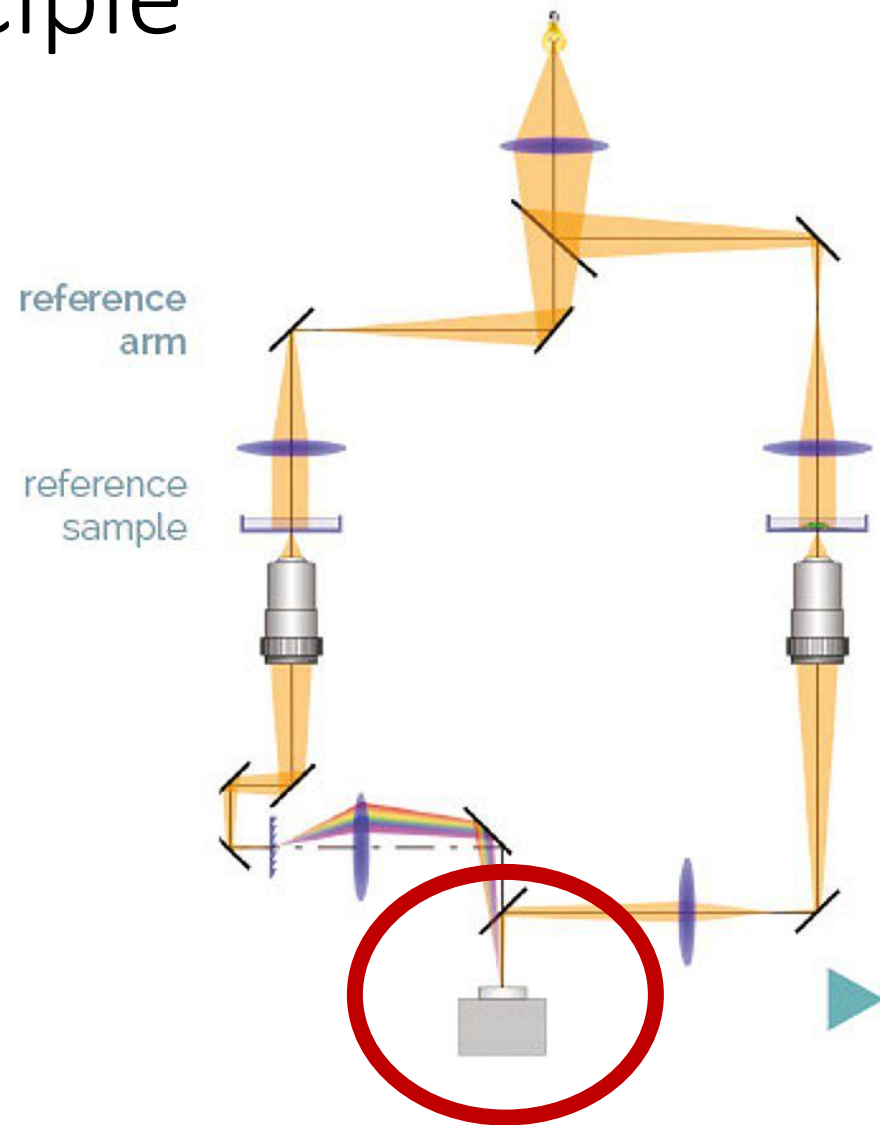
Red diode – 660 nm/10 nm – low photo-toxicity for cells



**Interference of two beams!**

# Principle

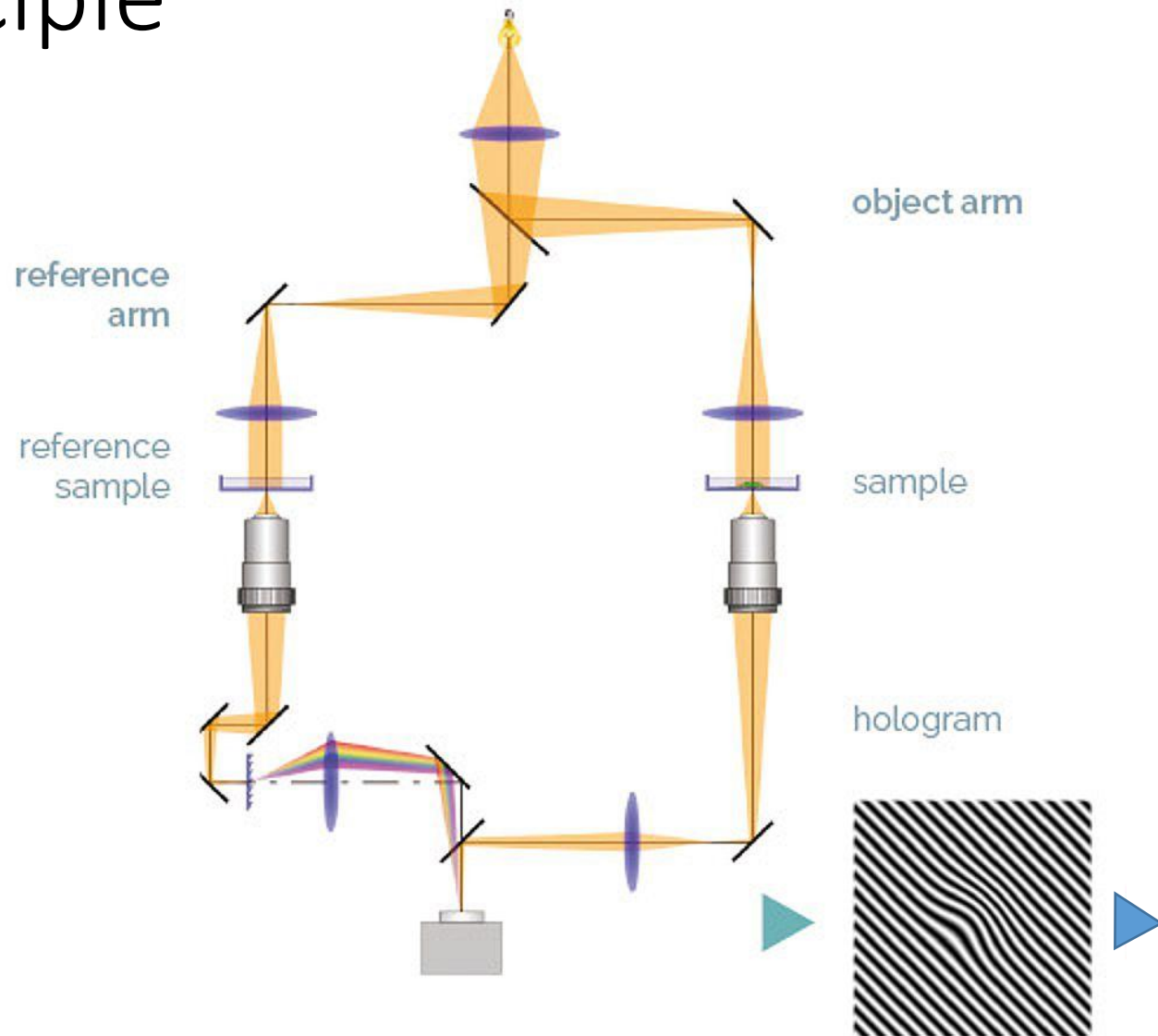
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**Interference of two beams!**

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Red diode – 660 nm/10 nm – low photo-toxicity for cells  
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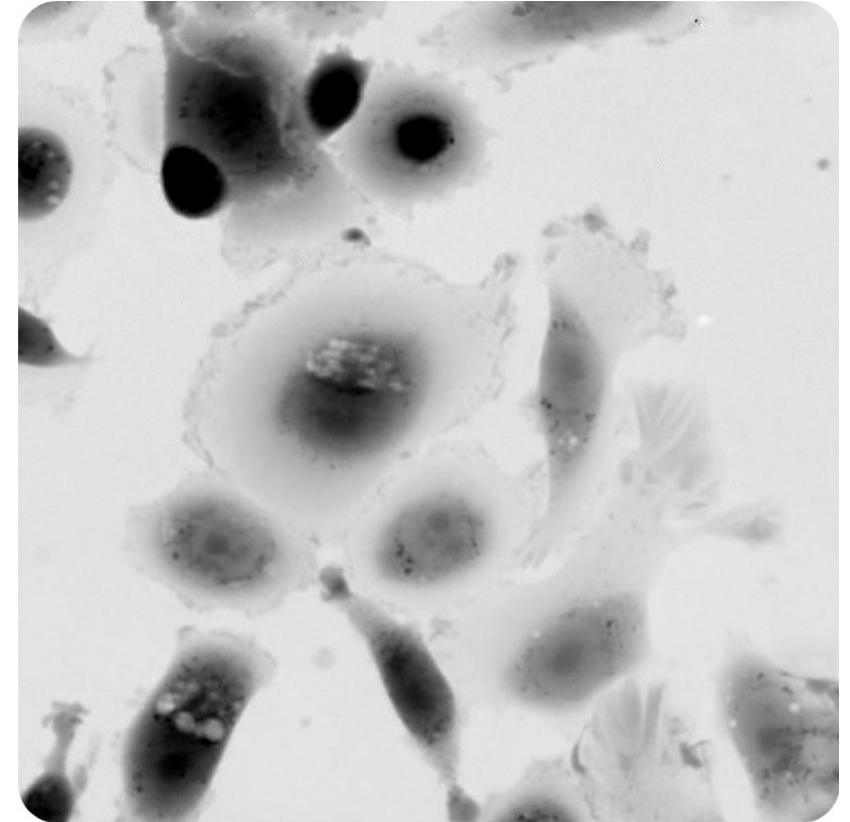
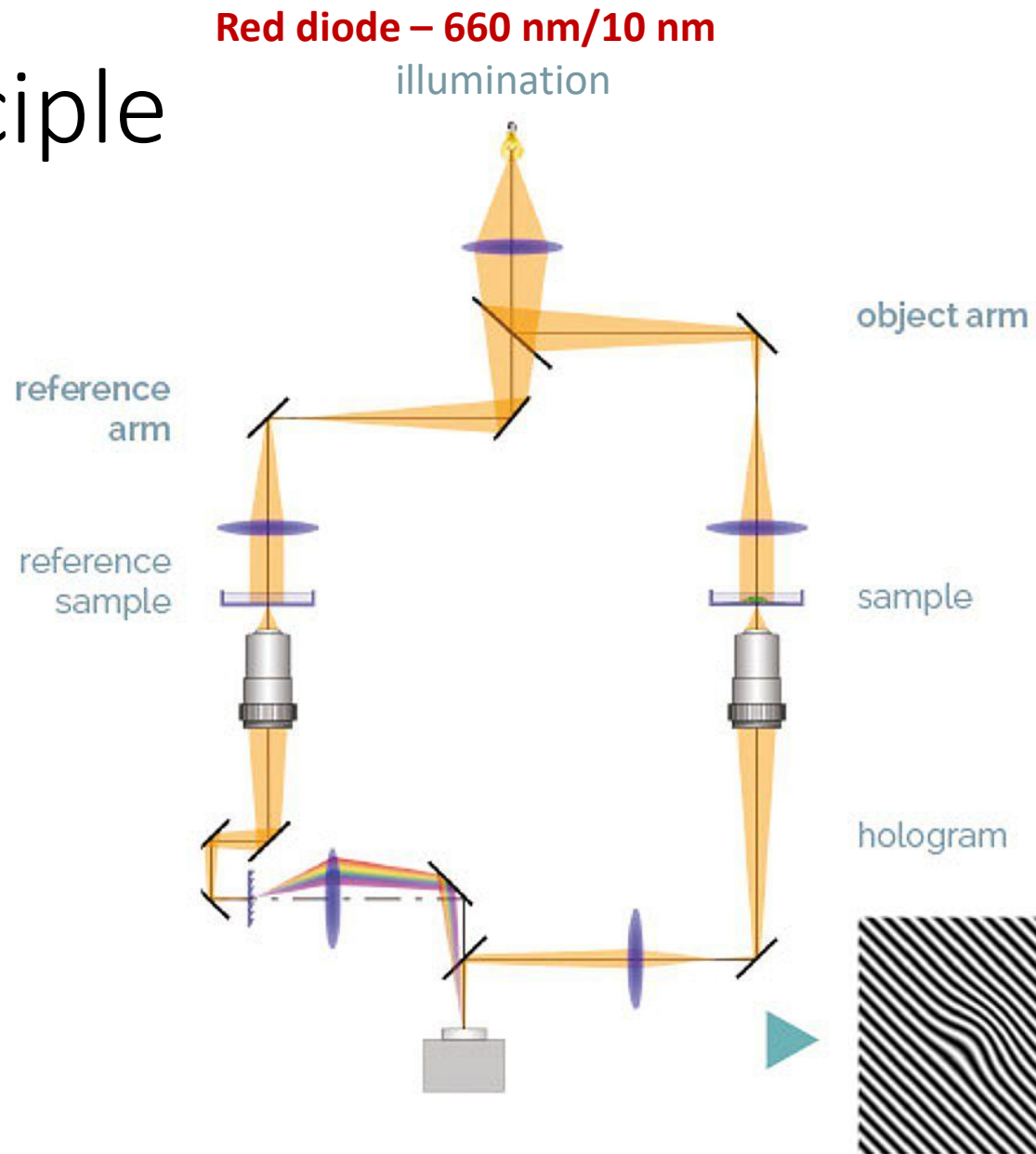


## Fourier Transform based reconstruction

- removing the carrier (stripes)
- extracting phase information



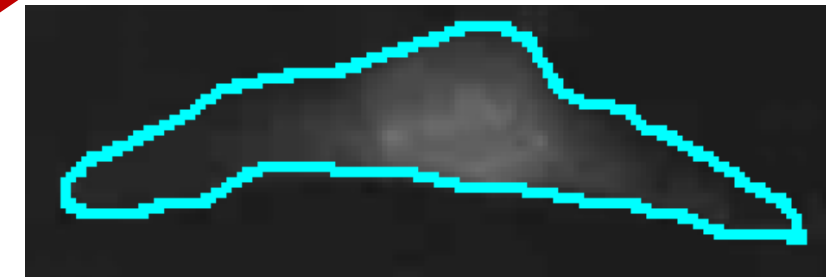
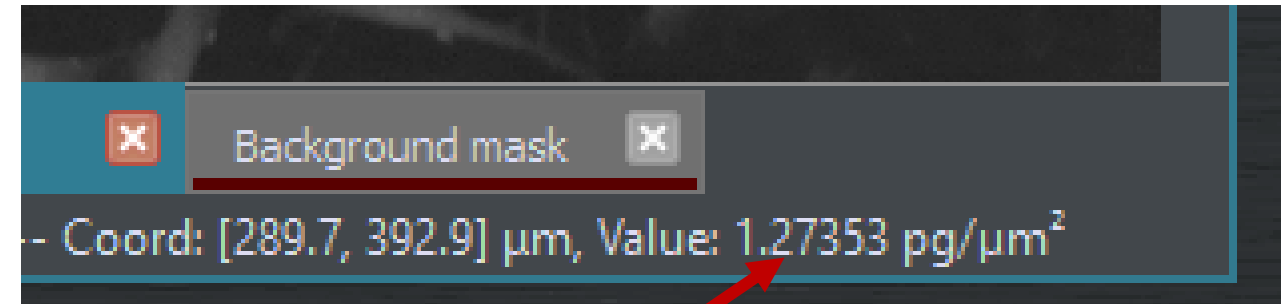
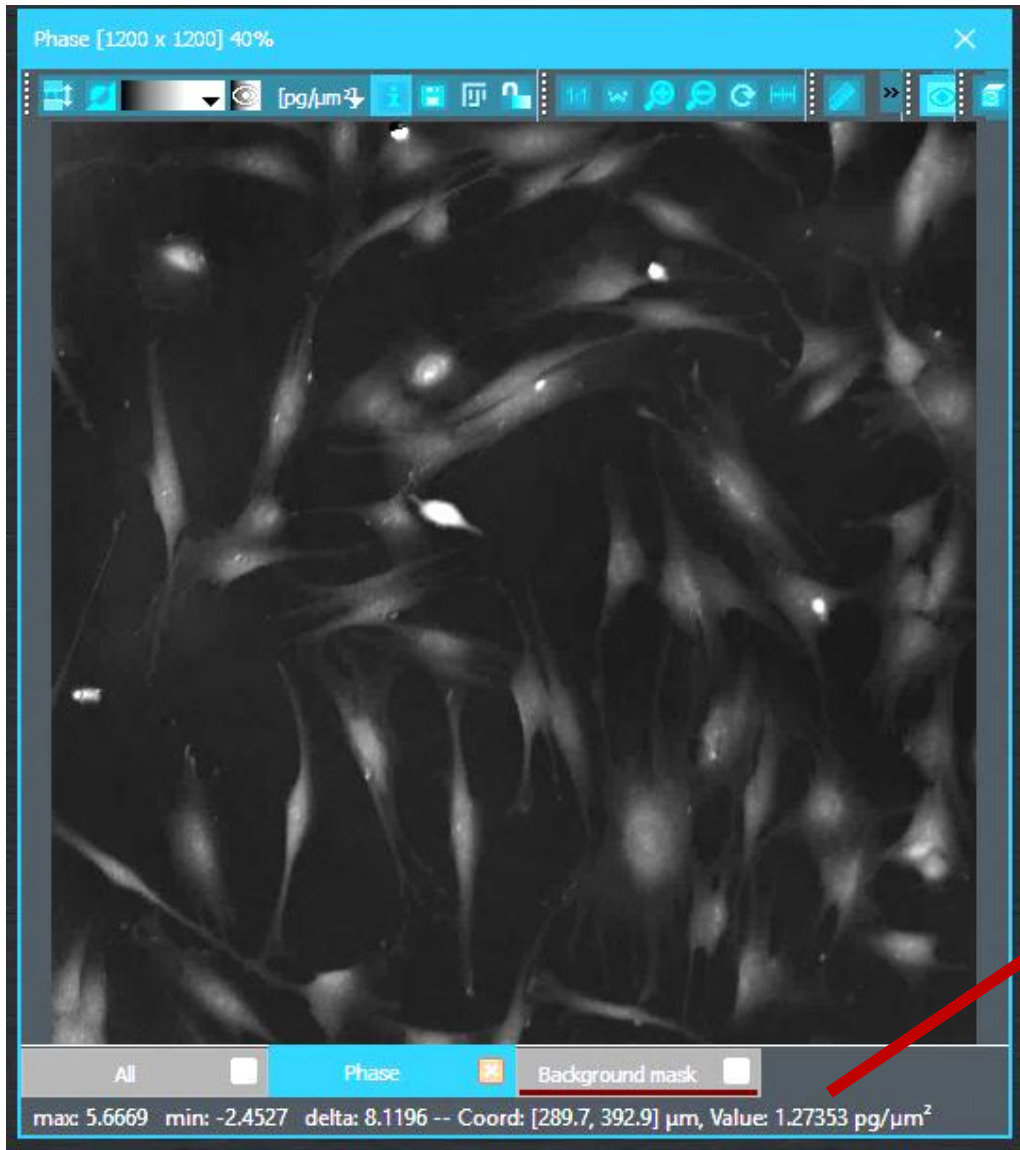
# Principle



## Fourier Transform based reconstruction

- removing the carrier (stripes)
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# Phase picture with cell dry mass density



By segmentation of cells – „mass“ of cells is computed!

$$\text{mass}_{\text{cell}} [\text{pg}] = \left( \sum_i (\text{pixels in a cell}) m_i [\text{pg}/\mu\text{m}^2] \right) \cdot \text{area}_{\text{cell}} [\mu\text{m}^2]$$

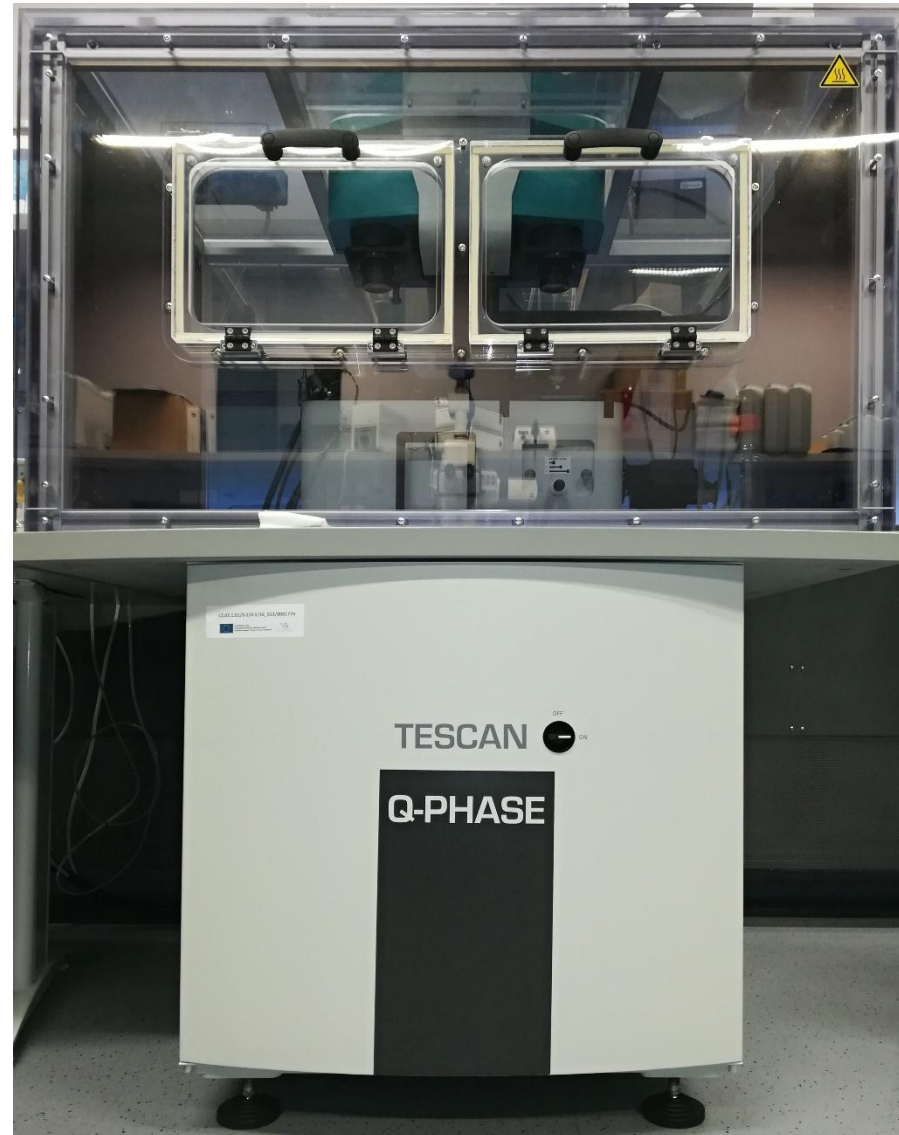
# Telight Q-PHASE Microscope



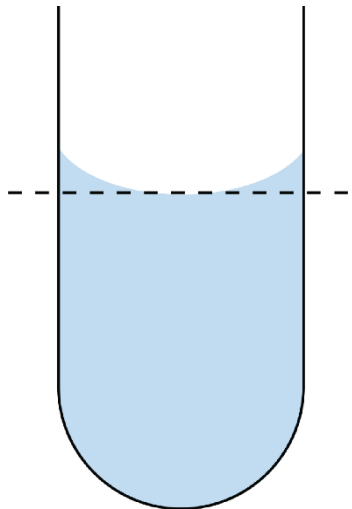
# Q-PHASE Microscope installed in LMCF IMG

Equipped with a microscope incubator

- Lowering **external light exposure**
- **Temperature, humidity & CO<sub>2</sub>** stabilization



# Sample preparation



water meniscus

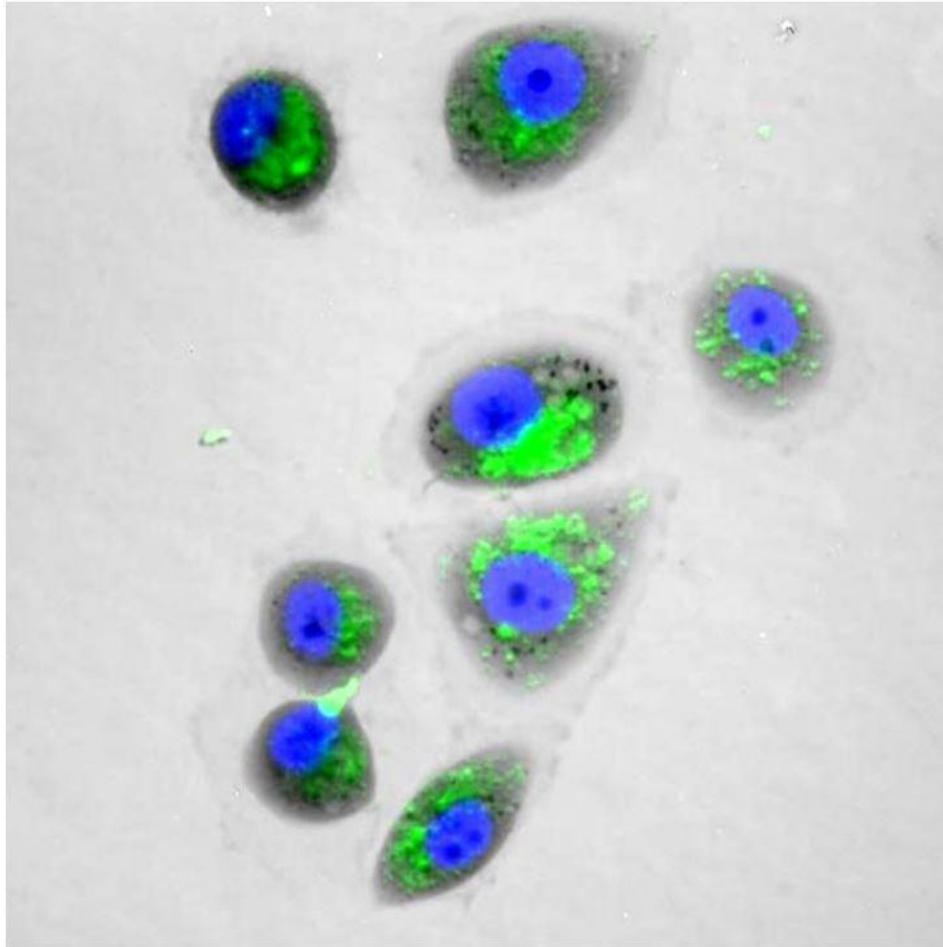


Ibidi slides with tunnels

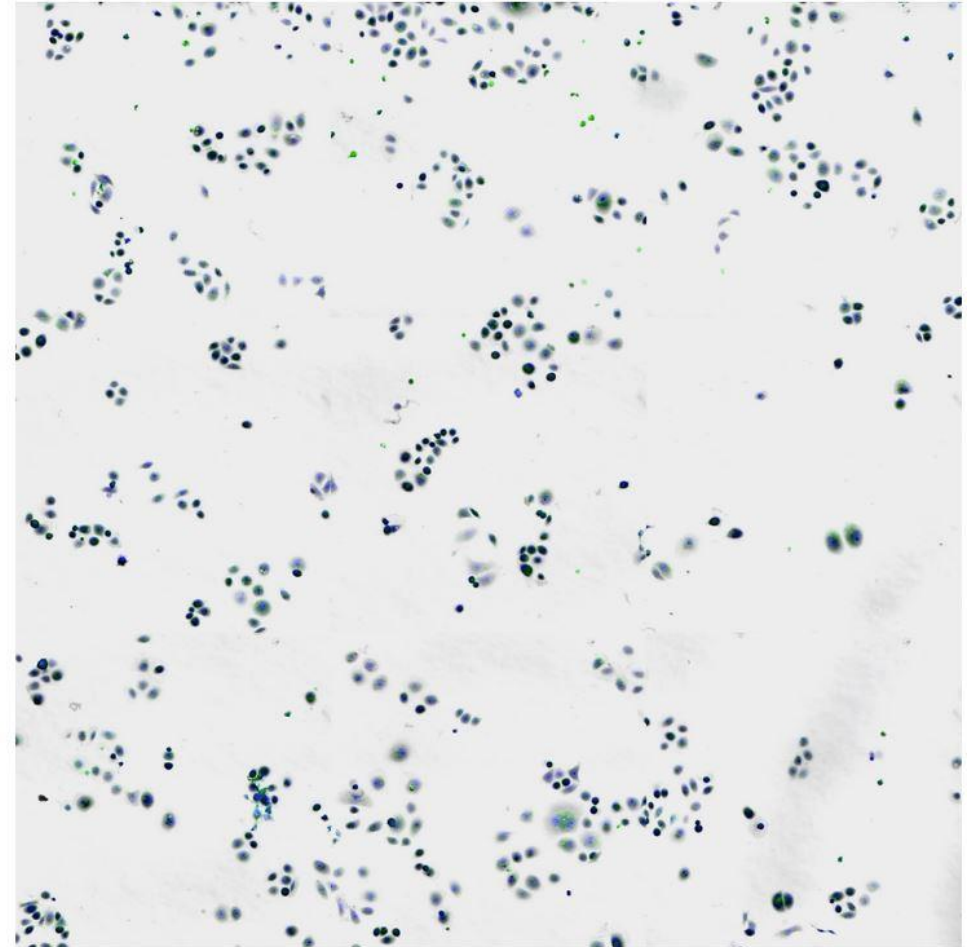




# Combining phase images with fluorescence

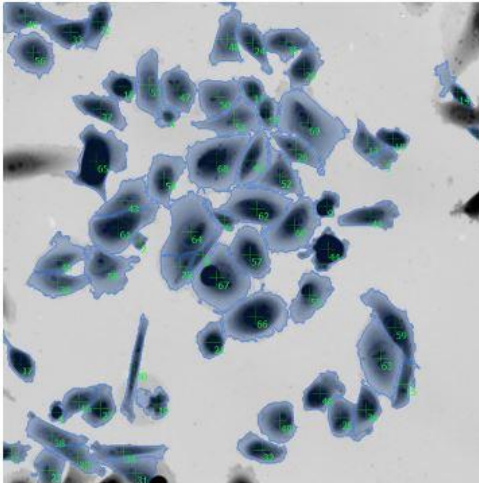


Autophagic human ovarian cancer cell line A2780 (fluorescence staining: Hoechst 33342, CytolD)  
(20x mag.)

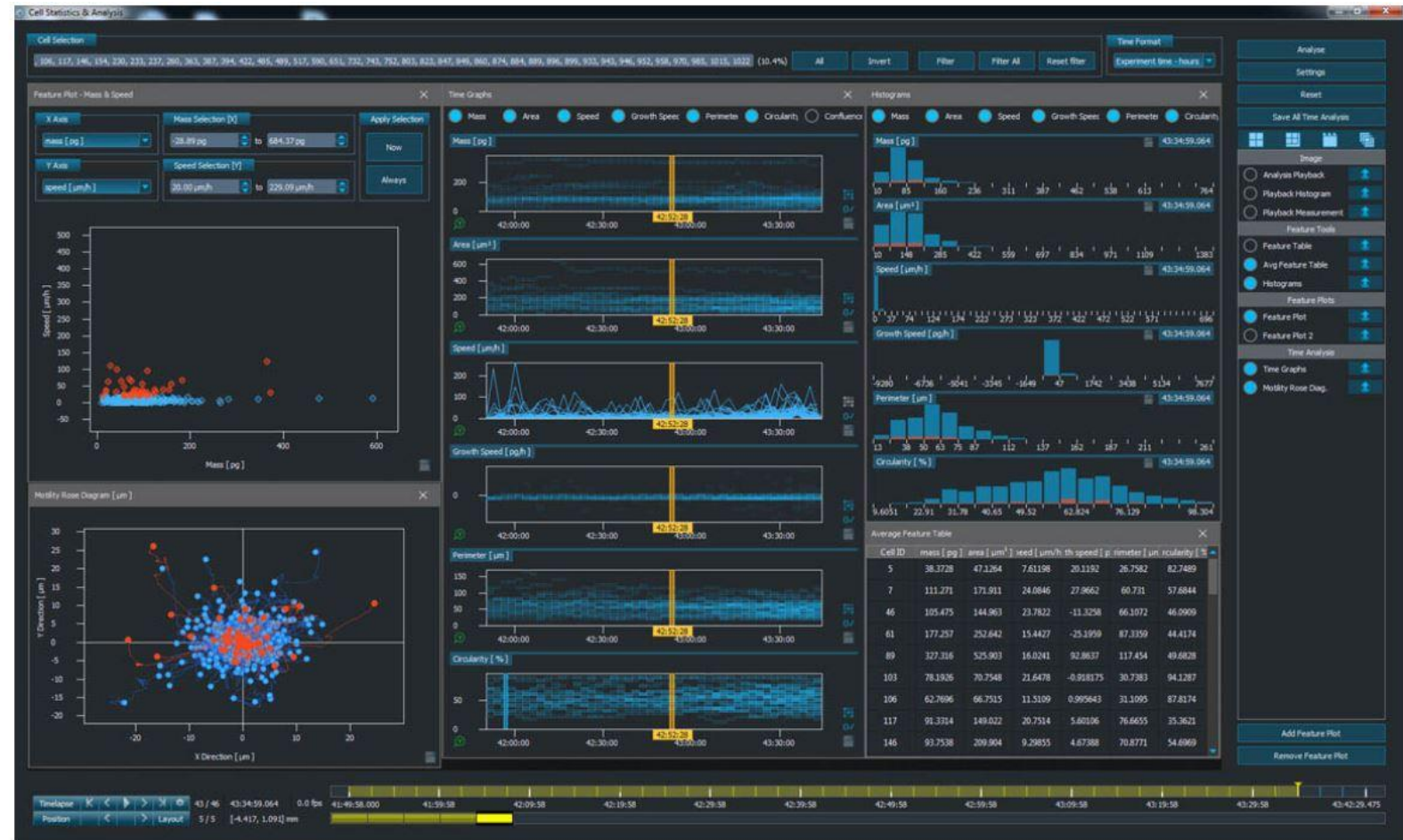


Human ovarian cancer cell line A2780 (fluorescence staining: Hoechst 33342, CytolD) (4x mag.)

# Advanced analysis of cells movement/growth/splitting/dying etc.



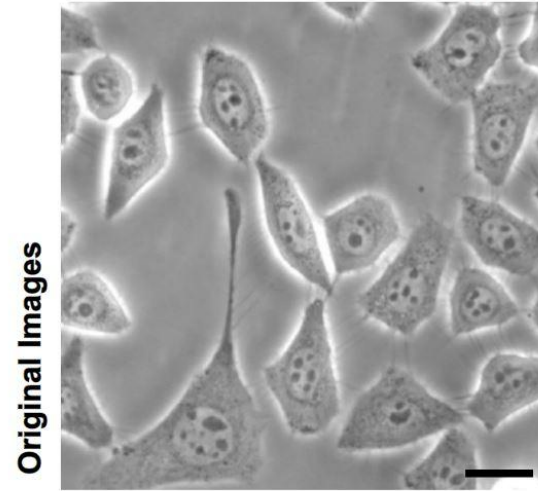
Segmented image ready for subsequent data mining



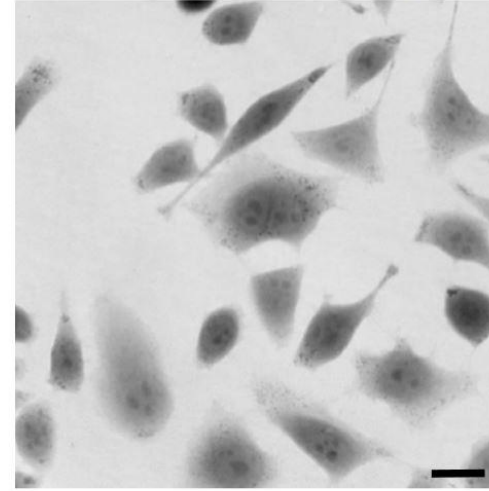
Automated cellular data analysis using Cell Analyser module

# Reducing halo-effect

**a** Phase Contrast Microscopy



**b** Holography Microscopy

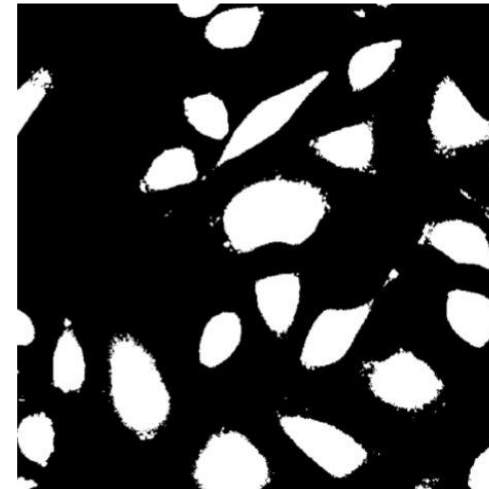


**c**

Thresholded Binary Images



**d**

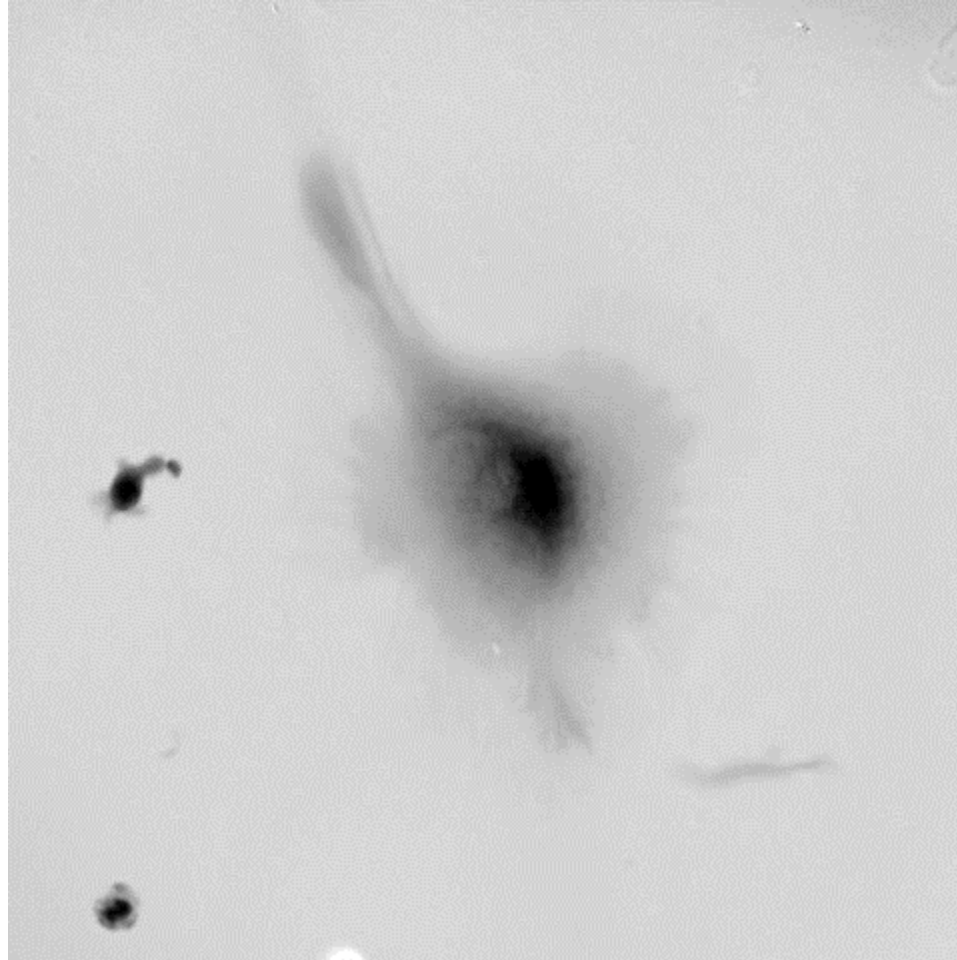




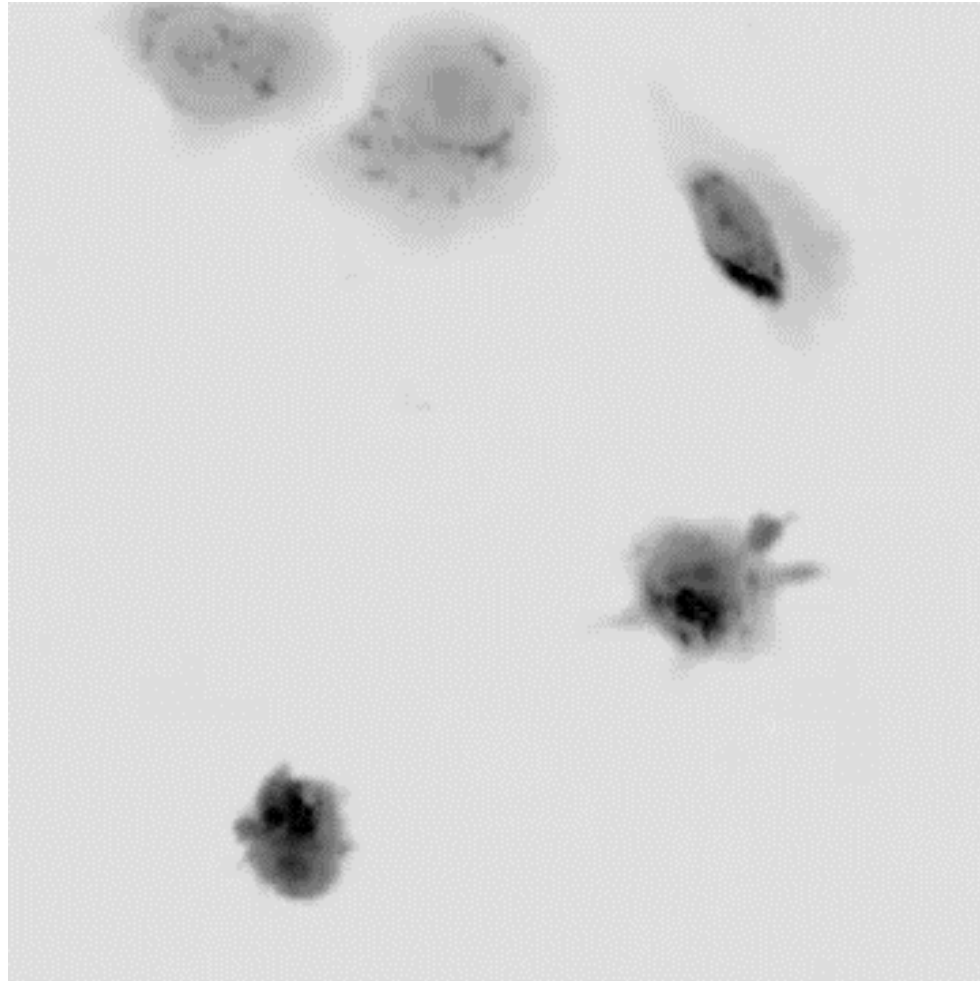
# Advantages 😊

- Live cell imaging
- **Low phototoxicity**
- Fast acquisition
- Resolution comparable to standard light microscopes ( $\sim 570$  nm, due to condensers used)
  - Internal parts of cells (nuclei, vacuoles, etc.) visible
- Transparent cells visible
- Good cellular contrast
- No image artifacts (e.g., halo and shade-off effects)
- Automatic segmentation of cells with high accuracy
- **Quantitative** (phase values can be recalculated to **cell dry-mass density** ( $\text{pg}/\mu\text{m}^2$ ))
- **Multimodal** – together with a fluorescence module

# Metastatic cancer cell – immune cell interaction (a T-cell invading a polyploid cancer cell)

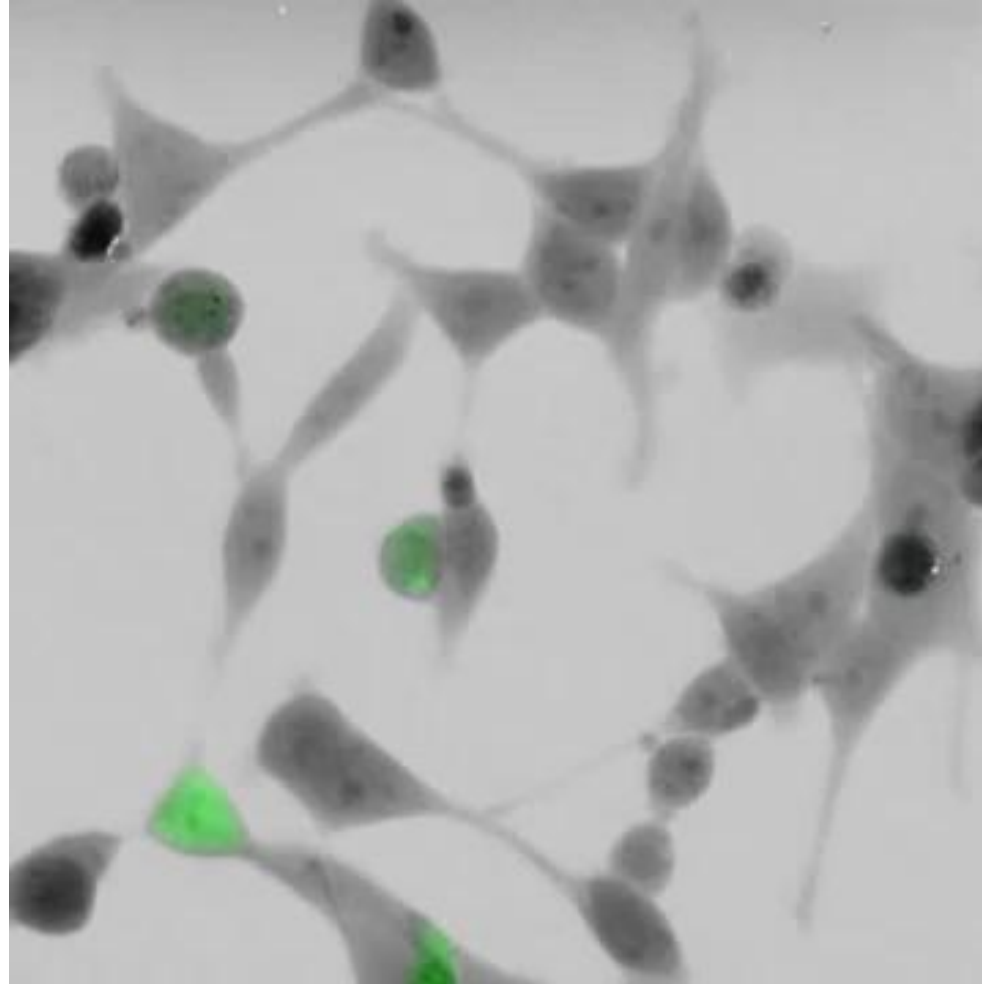


# Human embryonic stem cells division and proliferation in real-time at 20x magnification



<https://www.youtube.com/watch?v=2ZJ7hcZkF6s>

Observation of HEK293 cell population transfected with GFP-Vesicular Stomatitis Virus Coat Protein, 20x



<https://www.youtube.com/shorts/3mlXVjxxnN4>

<https://www.youtube.com/@telightco.4283/videos>

Thanks for your attention!