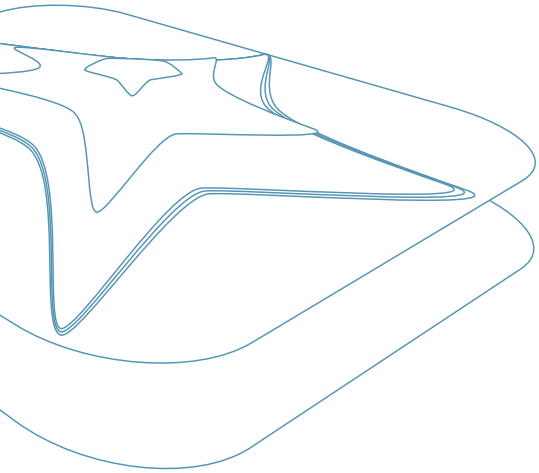


Real-time 3D monitoring of living biological specimens

DHM™ T1000 provides life sciences with new quantitative morphological measurements giving biologists a new criterion for biological interpretations with the following features:

- **3D visualization with nanometric axial resolution**
- **Real-time monitoring: cell swelling, health status, stimulation**
- **No contrast agents**
- **Non-invasiveness**
- **No phototoxicity**

DHM™ T1000 and Software Tools provided by Lyncée Tec make an ideal system to monitor and analyse biological specimens.

Non-invasive high-resolution imaging of living cells in their natural environment is a prerequisite to visualize biological processes induced naturally or artificially by chemical substances, electrical or thermal stimulations amongst others.

Conventional optical microscopes provide intensity images dependent on absorption (transmission mode) or reflectivity (reflection mode) characteristics of biological specimens under study. Classical phase contrast and Normaski's differential interference contrast are widely used in biology for the visualization of unstained transparent specimens. But they are not able to yield quantitative measurements of the phase shift induced by the specimen.

The DHM™ technology permits intensity measurements, as with a conventional microscope, but also the quantitative phase shift. The phase shift induced by the biological specimen depends on both the thickness (linked to morphology) and the refractive index (linked to the nature of the intracellular content). DHM™ system has important features making it an ideal instrument for dynamic studies of biological specimens:

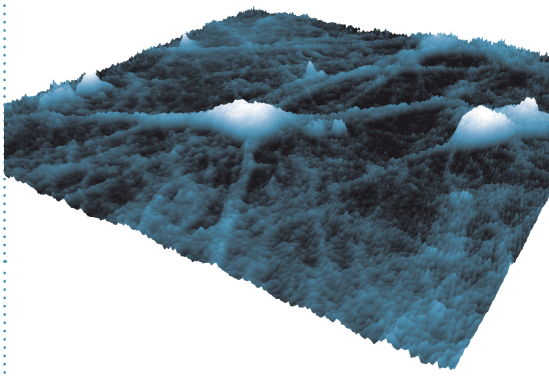
- No contrast agents
- Non-invasiveness
- No phototoxicity (irradiance 200µW/cm^2)
- Real-time measurements
- High acquisition rates up to 10'000 measurements per second
- Decoupling thickness and mean refractive index along vertical axis
- Lateral resolution down to 300nm
- Nanometric thickness resolution (10nm sensibility)

Moreover, digital tools implemented in the Koala Software allow efficient experimental processes and analysis both during the experiment and afterwards from the recorded data, such as:

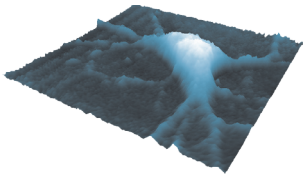
- Temporal monitoring
- Digital focusing: compensation for specimen displacement
- Digital compensation of optical aberrations: suppression of misalignment or displacement of the specimen holder

The DHM™ T1000 system is therefore an ideal tool for measuring quantitative dynamic morphological properties (refractive index, thickness) of biological specimens without special cell preparation (no contrast agents, no colorant) as demonstrated by publications and some results summarized on the next page. Although this list of possible application is not extensive, DMH has already been shown to be an effective tool to monitor and analyze:

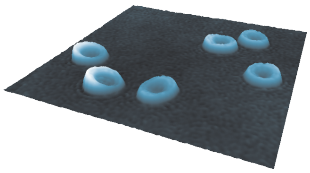
- Cellular swelling
- Cell health status
- Biological effects due to stimulation (drugs, electrical, thermal,...)



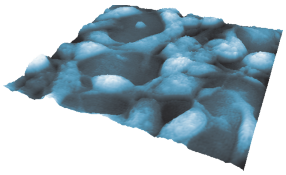
Quantitative 3D visualization of the phase shift of light transmitted through cortical mouse neurons net obtained with DHM™ T1000.



Living neuron cell
field of view 40x40µm^2



Erythrocytes
field of view 50x50µm^2



HEK 293 cells
field of view 100x100µm^2

lyncée tec DHM
PSE-A
1015 Lausanne
Switzerland
info@lynceetec.com
www.lynceetec.com

Hypotonic stress on neurons

A hypotonic shock on primary cultures of mouse cortical neurons is achieved by replacing a standard perfusion solution (229 mOsm/kg H_2O) by a hypotonic solution (144 mOsm/kg H_2O) reducing the extracellular osmolarity by 37%, which represents a relatively high stress for the neurons. As depicted in Fig. 1, the hypotonic solution produces a drop in the phase signal, which reaches a plateau after two minutes.

Such phase decrease remain difficult to be interpreted as a cellular swelling. To investigate this a priori paradoxical phase behavior, a patented decoupling procedure is applied to separately measure the mean integral refractive index along the vertical axis and the thickness of the neuronal cell bodies. The obtained morphometric images indicate clearly the expected hypotonic neuronal swelling. The hypotonic-mediated shape variations can be clearly seen in second row panel of Fig. 2. One should note a nonhomogeneous neuronal swelling, weaker in some central domains of the cell body (green-yellow region in second row panel), proportional to the cellular thickness before the hypotonic shock. The hypotonic shock induces a mean integral refractive index decrease. This decrease is consistent with a hypotonic water influx, resulting in a dilution of the intracellular protein concentration, the cellular component which largely determines the mean integral refractive index value.

Erythrocyte membrane

The spontaneous temporal fluctuation of the erythrocyte membrane is evaluated by measuring the phase deviation during a 10s period (13 frames/s) for two fixed (left images in Fig. 3) and two living (right images in Fig. 3) erythrocytes. The central region of the fixed ones (inside white circles) fluctuates as much as a reference region taken outside the cells (inside white rectangles) (0.95° and 0.97°, respectively). In contrast, the central region of the living ones fluctuates significantly more than the outside cell regions (1.8° and 1.1°, respectively). The fluctuation in the central regions depends on the noise (measured outside the cell) and on actual membrane fluctuation. Subtraction of this noise contribution allows measuring the actual membrane fluctuation which corresponds to a thickness fluctuation of ~40nm. This measurement can be performed to study the influence of various drugs on the membrane fluctuations for example. Indeed, these measured fluctuations depend on the fitness of the cell and can be used to assess the health of a cell during different experimental conditions.

Conclusion

The DHM™ T1000 is an ideal instrument for life sciences because it performs quantitative and accurate measurements of biological specimen characteristics such as thickness and refractive index. Moreover, the effects of stimulation (drugs, electrical stimulation) can be monitored with easy-to-use software tools helping biologists to understand and to interpret the biological specimen mechanisms.

References

B. Rappaz et al., "Measurement of the integral refractive index and dynamic cell morphometry of living cells with digital holographic microscopy", Opt. Express 13, 9361-9373 (2006)

P. Marquet et al., "Digital holographic microscopy: a noninvasive contrast imaging technique allowing quantitative visualization of living cells with subwavelength axial accuracy", Opt. Lett. 30, 468-470 (2005)

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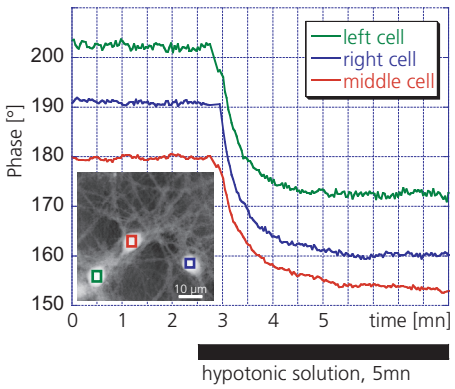

Figure 1: *Real-time monitoring of the phase signal of 3 neurons observed during a hypotonic shock. Inset: quantitative phase contrast image of the monitored neurons. The phase mean values of the colored rectangles are plotted versus perfusion time. The black bar denotes perfusion of the hypotonic solution for 5 minutes.*

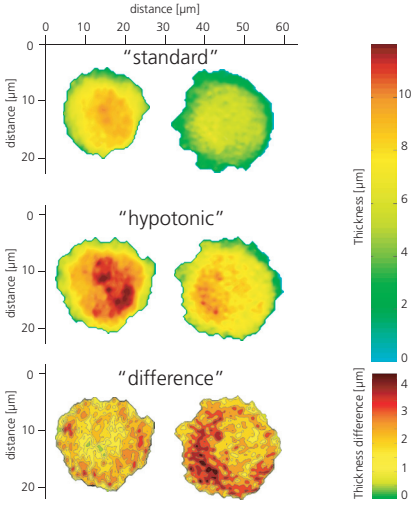

Figure 2: *Morphometry of 2 cell bodies before (first row) and 3 minutes after the onset (second row) of a hypotonic shock. Here the z-axis (cellular thickness) is expressed in micrometers. These values are obtained using the results of the decoupling procedure. Third row: color-coded distribution of thickness variations resulting from the subtraction of the "standard" image to the "hypotonic" image.*

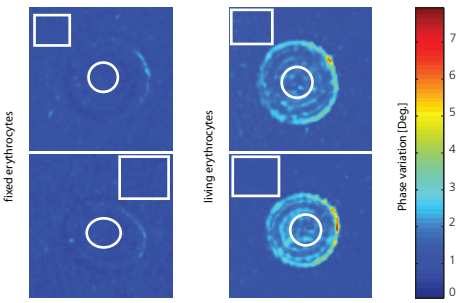

Figure 3: *Temporal phase deviation map for two fixed (first row) and two living (second row) erythrocytes. White circles and rectangles indicates respectively the central region of cells and the outside reference regions.*