

Living cells analysis: shape and structure measurements

By providing accurate specific quantitative optical parameters, impossible with standard optical microscopes, the DHM™ technology is a new efficient cellular imaging tool allowing :

- Visualization of transparent specimens without contrast agent
- Shape and structure measurements with nanometric resolution
- Real-time analysis of biological specimen dynamics
- Cellular optical properties related to protein concentration

The DHM™ system provides life sciences with a new efficient criterion for biological specimen analysis and recognition.

Real-time analysis of living biological specimens is of great importance today in various domains such as pharma, health care, national security, food industry (pollen allergies, pandemic diseases, biological terrorism or bacterial contamination).

Conventional methods of inspection require specific labelling of biological structures, recognition processing of images obtained from conventional microscopes or contrast enhancing techniques including phase contrast (PhC) and Nomarski's differential interference contrast (DIC) microscopes. Specific labeling processing usually involves time consuming complex tasks often associated with undesirable side effects (photobleaching and/or photodynamic damage as far as fluorescent probes are concerned). On the other hand, conventional microscopes provide information about the specimen (2D size, optical absorption or reflectivity, color) that is very often not enough for an accurate cellular dynamics analysis. The widely used PhC and DIC techniques suffer from optical artifacts making the interpretation of their images in terms of the corresponding cellular structure difficult. In addition, the focalization in conventional microscopes has to be adjusted to compensate for specimen movements or to select a specimen

placed in another plane.

Within this framework, the DHM™ T1000, thanks to its ability to perform an accurate quantitative measurement of the wave front deformation induced by the observed specimen on the transmitted light, allows efficient cell structure and dynamics analysis with the following advantages:

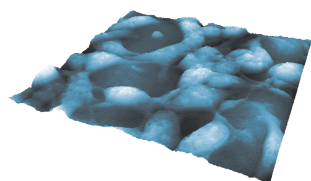
- 3D visualization of cell structure and dynamics with a sub-wavelength axial resolution
- Cellular optical properties related to the intracellular protein concentration: intracellular refractive index
- No contrast agents required
- Non-invasiveness
- No phototoxicity (irradiance 200μW/cm²)

Moreover, digital tools allow efficient real-time image analysis as well as powerful post-processing of the recorded data such as:

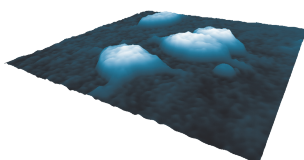
- Temporal monitoring
- Digital focusing
- Digital compensation for optical aberrations

The digital focusing allows, without any mechanical movement, to focalize any plane along the optical axis to compensate for specimen displacement during the experiment or to focalize different organisms at different depths in a solution. The automatic compensation for aberrations suppresses misalignments or deformations induced by the solution chamber during the experiment.

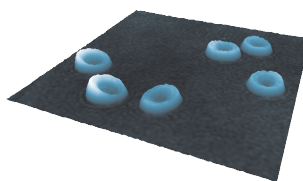
The DHM™ system is therefore an outstanding tool for the analysis of the structure and dynamics of biological specimens, for example micro-organisms, cells or pollen particles, as shown in the next page.



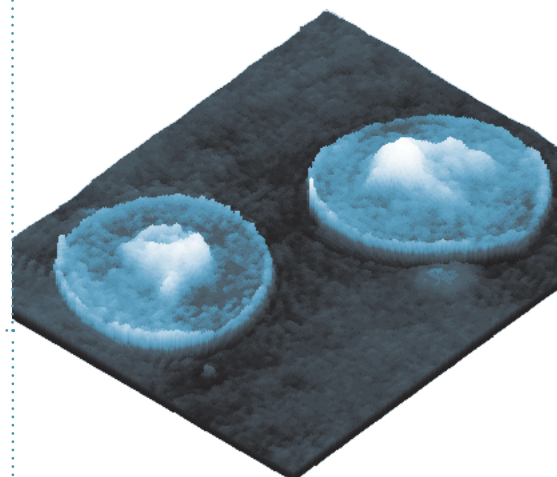
HEK 293 cells
field of view 100x100μm²



Undifferentiated PC12 cell line
field of view 64x64μm²



Erythrocytes
field of view 50x50μm²



3D visualization of quantitative phase shift of light transmitted through two yew pollen particles obtained with DHM™ T1000.

Quantitative phase contrast

Images of the same living neurons in culture obtained with a DHM™ T1000, phase contrast (PhC), and Nomarski's differential interferometric contrast (DIC) microscopy are compared in Fig. 1. The dark PhC image (a) makes the neuronal processes particularly visible, but suffers from well-known optical artifacts, namely, the halo (bright zone surrounding the neuronal body and processes in the background) and shading off (phase increase in the central part of the neuronal body, making it appear brighter than the thin neuronal processes). The predominant feature of DIC images (b) is their three-dimensional appearance as a result of virtual lateral illumination, or the shadow-cast effect. The contrast of DIC images is, therefore, not symmetrical and varies proportionally to the cosine of the angle made by the azimuth of the object and the direction of wave-front shear. Finally, DHM™ T1000 allows quantitative phase contrast and 3D visualisation of living neurons (c). Furthermore by using a patented decoupling method or assuming a constant and homogeneous cellular refractive index, one can measure, with subwavelength resolution, the thickness of the cell body during the neuronal processes.

Cell type sorting: pollen grains

Pollen particles samples, with size ranging between 20µm and 30µm, were examined. Figure 2 presents typical examples of amplitude (first row) and phase (second row) images for

three different pollen types: birch, yew and dandelion. The complementarity of the two kinds of image, phase and amplitude, is clearly visible on this figure: the amplitude images are related to the absorption of light by the sample, whereas the phase images are directly proportional to the optical path length of the sample, i.e. sensitive to both refractive index and thickness changes in the sample. In the last row of Fig. 2, typical profiles of the different pollen particles types are shown. It shows clearly the uniqueness of the phase signal for each pollen type, as the refractive index can be different for each cell part (membrane, core, cytoplasm) as well as for each pollen type.

The negative phase difference visible on the birch pollen profile (the depletion on each side

of the cell) can be explained by a refractive index in the membrane smaller than the extra-cellular environment (a liquid with a refractive index about 1.3). In contrast, on the yew pollen profile, small spikes on both sides of the cell are due to a refractive index in the membrane greater than both cytoplasm and extra-cellular environment refractive indexes. On the dandelion pollen profile, neither depletion nor spikes are visible, this signifies that the refractive indexes in the membrane and in the cytoplasm are about the same value, greater than the extra-cellular environment. Their shapes, different due to specific cells architectures, and their ranges, from 200° and 250° for respectively the birch and the yew cells to 600° for the dandelion cell, can be used to achieve cell type sorting.

Conclusion

The DHM™ T1000 is an ideal instrument allowing non-invasive analysis of biological specimen structure and dynamics providing, thanks to the quantitative phase measurements, accurate information concerning both the 3D morphology and the protein concentration related refractive index.

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)

<http://www.lynceetec.com/downloads/>

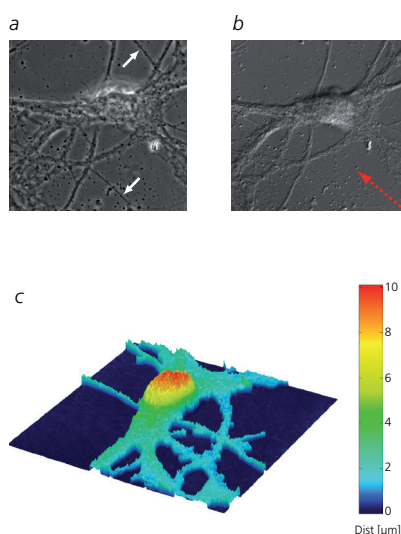


Figure 1: Images of a living mouse cortical neuron in culture: a) dark PhC image; b) DIC image; c) perspective image in false colors of the phase distribution obtained with the DHM™ T1000.

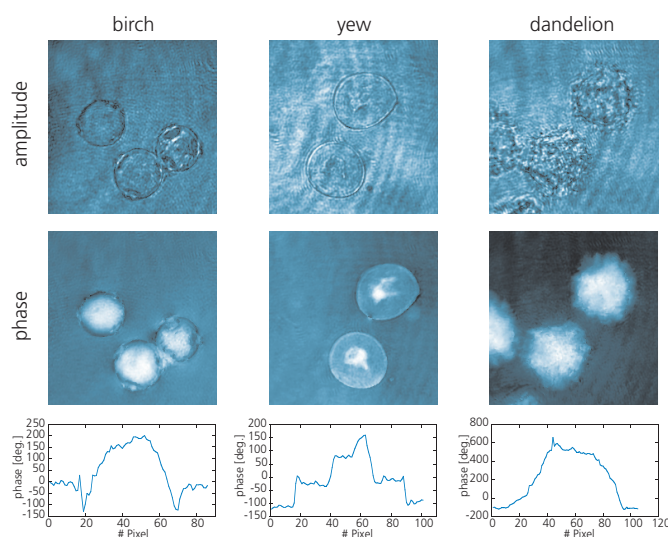


Figure 2: Different pollen particles imaged with transmission DHM™ T1000 and profiles extracted from phase images.