

Digital Holographic Microscopy (DHM)

3D Real-Time Optical Imaging at the Nanometer Scale

Digital Holographic Microscopes (DHM) enables strictly noninvasive visualisation of unstained transparent and partially reflective specimens, in real time, by providing simultaneously amplitude and phase changes of a light wave transmitted or reflected. They are used for characterisation of samples at the nanometer scale, for quality control on production line, and for dynamical analysis of biological specimen and micro systems.

The Digital Revolution in Holography

Light diffraction by a sample modifies both intensity and phase of the illuminating wave. As any photosensitive media responds only to the light intensity, in 1948 Denis Gabor invented a way to encode the phase information as an intensity modulation: the "hologram" [1]. Digital Holographic Microscopes (DHM), as shown in figure 1, digitally implement this powerful concept of holography. With the present performances of computers and the developments of digital cameras, holograms can be digitally recorded and numerically reconstructed to provide simultaneously: (1) the phase information with an axial resolution below 1° of the wave phase (less than one nanometer for homogeneous reflective samples), and (2) intensity images, as obtained by conventional optical microscope. Both images are defined with a diffraction-limited resolution in the transverse (Oxy) plane and are "reconstructed" from the hologram in real time:

more than 15 reconstructions per second for 512×512 pixels holograms with a standard personal computer.

The strength of the DHM lies on the use of the so-called off-axis configuration in particular [2], illustrated in figure 2, which enables to retrieve the 3D phase and intensity images of the observed object by numerical reconstruction of a single hologram, which comprises the whole information necessary to reconstruct phase and intensity images, and which can be acquired in a few ten of microseconds.

Cellular Dynamics Revealed by DHM

In biology, most microscopy specimens, in particular living cells, are transparent and differ only slightly from their surroundings in terms of absorbance and colour, thus providing a modest change in the amplitude of the light wave. Examination of such transparent specimens, which have the capacity to alter the phase of the detected light wave and are called phase



Fig. 1: DHM configured in reflection by Lyncée Tec SA. It allows 3D real time measurement at the nanometer scale of biological and material sciences samples.

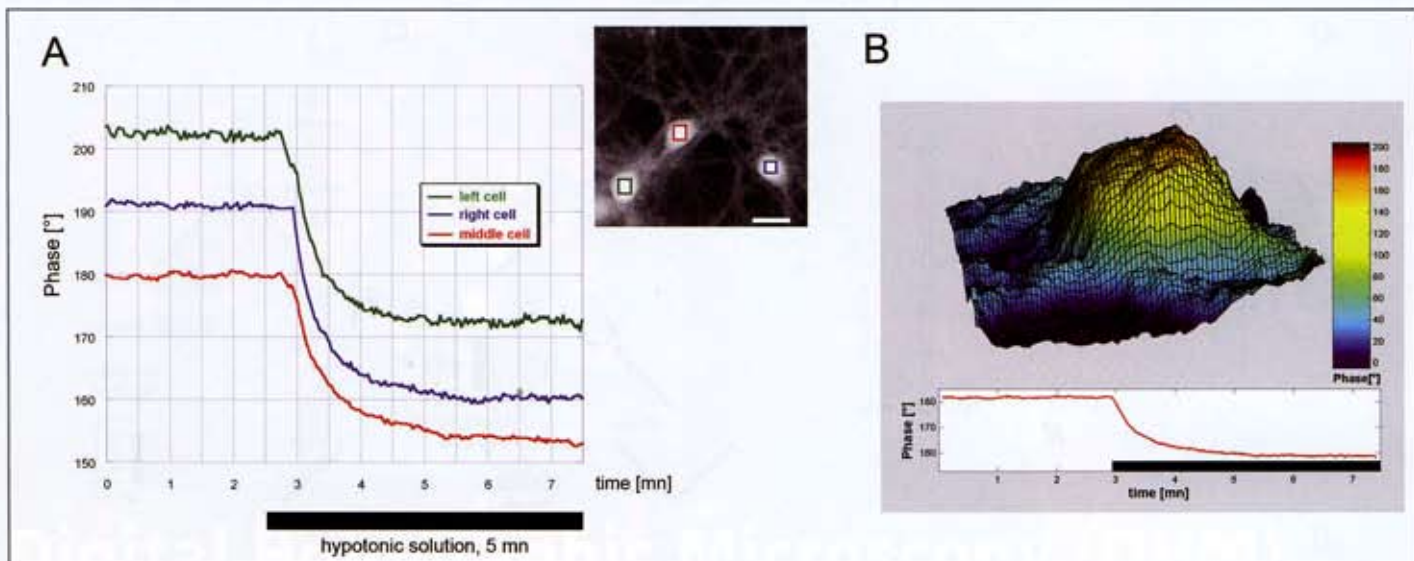


Fig. 3: Neurons in culture during a hypotonic stress (a) Real-time monitoring of the phase signal of three neurons observed during a hypotonic shock. Inset: quantitative phase contrast image of the monitored neurons. The phase mean values of the coloured rectangles are plotted versus perfusion time. The black bar denotes perfusion of the hypotonic solution for 5 minutes. Scale bar in the inset image: 10 μ m. (b). Morphology and temporal evolution of the spatial distribution of phase of the central neuron on the inset image in (a) when the hypotonic solution is perfused [3].

dynamic characterisation of opto electronically or magnetically active samples, micro optics shape and surface specification [6], including high NA optical components [7]. A few of these applications are illustrated in figure 4.

Ready for New Applications

DHM are new 3D measurement instruments providing diffraction limited lateral resolution and nanometer scale

vertical resolution. They provide unrivalled possibilities in term of speed and ease of use. They open new fields of research and new applications in both Life and Material Sciences.

Acknowledgements

The development of the technology has been supported by Swiss government through CTI grants TopNano 21 #6101.3 and NanoMicro

#6606.2 and #7152.1. Systems are commercialised by Lyncée Tec SA (www.lynceetec.com).

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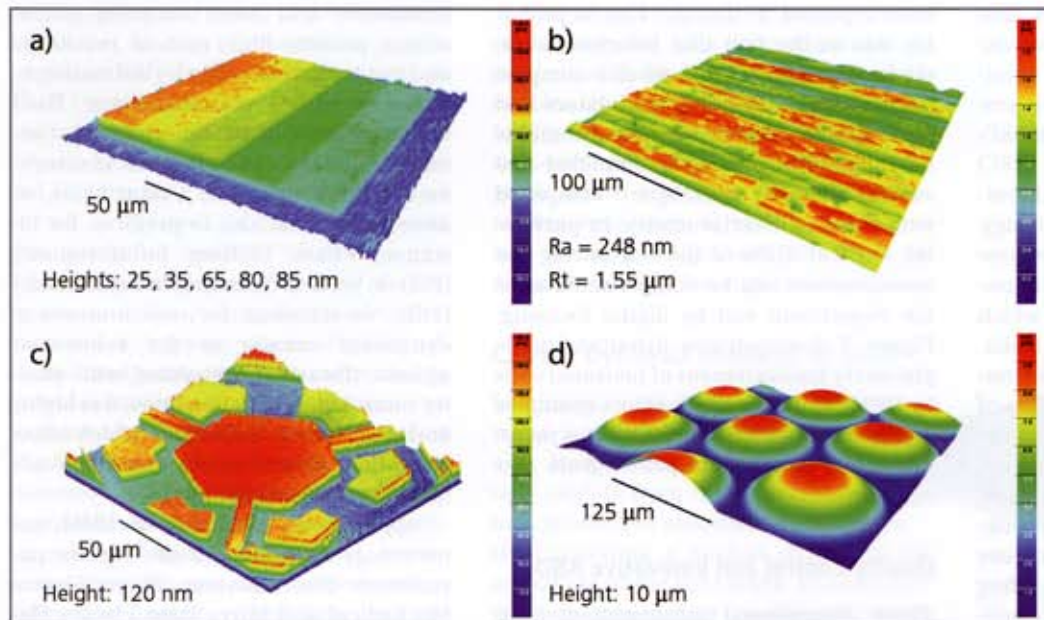


Fig. 4: (a) Optical topography at the nanometer scale, (b) Roughness parameters surface measurements (c) MEMS dynamical response analysis, and (d) micro lenses shape and surface measurement.

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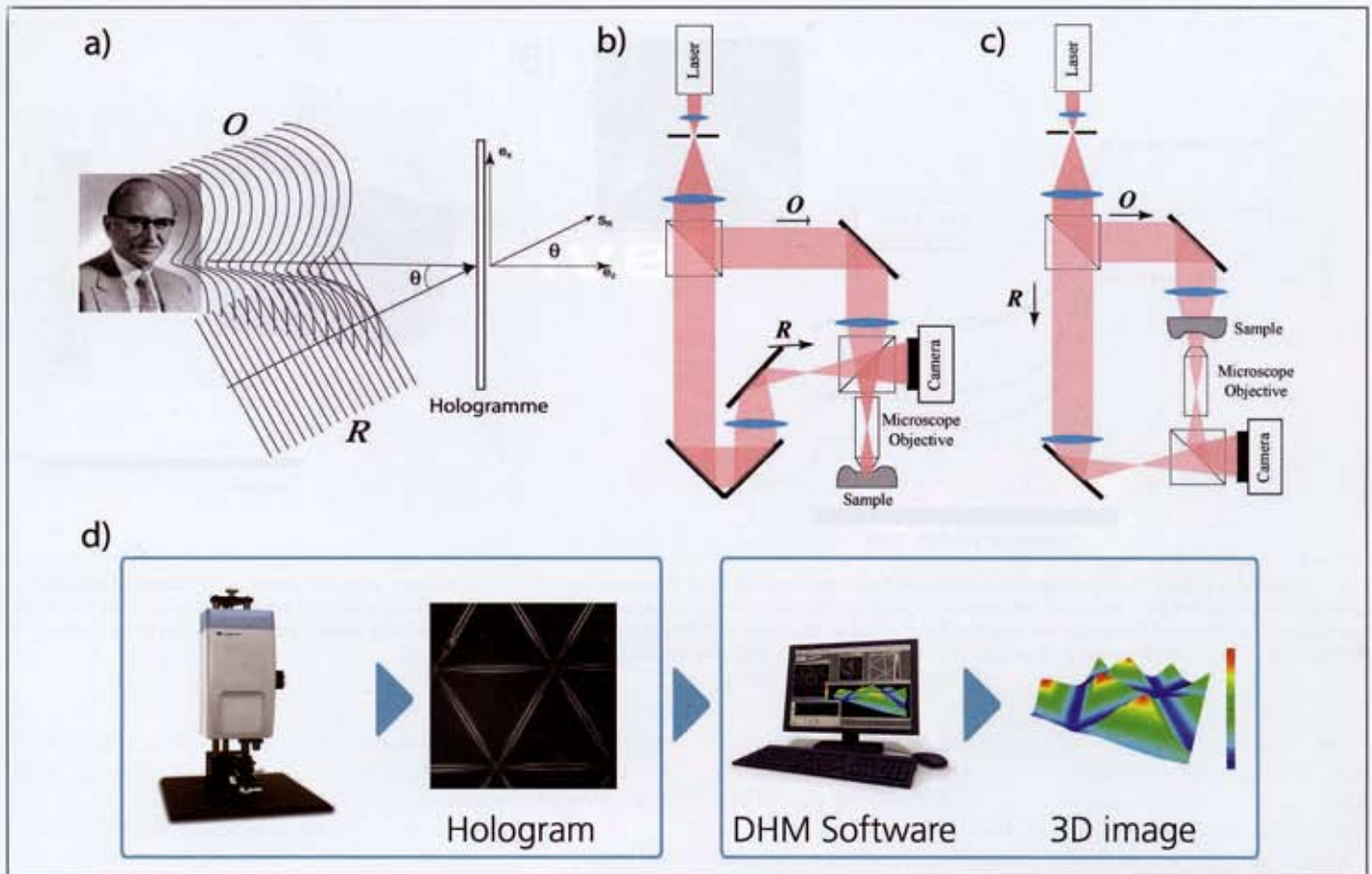


Fig. 2: (a) Recording principle of off axis holograms. There are a few degrees (angle θ) between the reference (R) and the object beams (O). This enables to reconstruct the information using a single hologram acquisition. On-axis holography (i.e. $\theta=0$) requires acquisition of several holograms. The portrait is the one of Dennis Gabor, Nobel Prize of physics, for its discovery of Holography. (b) Optical set up for reflection DHM: the source beam is first separated into two parts: a reference beam and an object beam. The object beam illuminates the sample. The light diffracted by the sample is collected by the microscope objective and is combined with the reference beam to form a hologram on the camera. (c) Optical set up for transmission DHM: the source beam is first separated into two parts: a reference beam and an object beam. The object beam illuminates the sample. The light diffracted by the sample is collected by the microscope objective and is combined with the reference beam to form a hologram on the camera. (d) DHM principle: once the hologram has been captured by the microscope, it is numerically interpreted to provide phase and intensity images of the sample.

objects, has led to the development of optical contrast-enhancing imaging techniques. Among the numerous modalities of contrast enhancing techniques that have been developed for non invasive visualisation of unstained transparent specimens, phase contrast (PhC), initially proposed by Zernicke as well as Normarski's differential interference contrast (DIC) are available for high-resolution light microscopy and are widely used in biology. These two contrast techniques allow one to transform phase information into amplitude or intensity modulation, which can be detected by photosensitive media. In addition to the above-mentioned microscopy techniques, the availability of lasers, modulators, and sophisticated detectors have promoted the development of various interferometric techniques. Unlike the PhC and DIC microscopy techniques, interferometric techniques present the great advantage of yielding quantitative measurements of parameters, including the phase distribution produced by transparent specimens [3].

However, whereas interferometric techniques are widely used in material sciences, only a few applications have been reported in biology. This is probably due to the fact that interferometric measuring setups often involve complex laser systems, including modulators and piezo-driven mirrors, requiring complex handling. DHM provide a simplified and easy-to-operate technique compared with classical interferometry. In particular, vertical drifts of the cell during the measurement can be compensated after the experiment end by digital focusing. Figure 3 demonstrates dynamical morphometry measurement of neuronal cells by DHM [4]. Other applications examples are the observation of erythrocytes membrane fluctuations and pollen grain recognition.

Quality Control and Innovative R&D

Three dimensional measurement with nanometer scale resolution along the three axis are provided by Atomic Force

Microscopes (AFM). Their limitation is the time required for the measurement and the small area measured. The interferometers and some scanning microscopes provide high vertical resolution and are limited laterally by diffraction to a few hundreds of nanometers. Their limitation is the need of mechanical scanning, either to cover the full measurement field of view with a laser beam, or along the optical axis to perform, for instance, Phase Shifting Interferometry (PSI) or Vertical Scanning Interferometry (VSI). Nevertheless, for measurement of dynamical sample or for robustness against vibrations associated with quality control on production lines, it is highly desirable to use techniques which allow a full-field measurement in a single acquisition, as provided by DHM.

Applications examples of DHM are metrology at the nanoscale, surface parameters determination, Micro Electro Mechanical and Micro Opto Electro Mechanical Systems (MEMS and MOEMS) dynamical response investigation [5], dy-