CCHM – a novel technique of digital holographic microscopy for in vitro study of cell behaviour

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Coherence Controlled Holographic Microscope (CCHM) [1] (Fig. 1a) has been developed. The difference from standard Digital Holographic Microscope (DHM) lies in an opportunity to choose illumination with arbitrary low coherence of light. This means that holographic imaging is achieved without laser light and the use of standard illumination of wide-field microscope is possible. As a consequence CCHM images are of low noise, deprived of speckles and lateral resolution is improved by a factor of 2 compared to ordinary DHM. These achievements further improved Quantitative Phase Contrast (QPC). QPC from cell biology point of view represents the main methodical contribution of DHM. It ascribes a numerical value of object beam phase shift in nm as compared to reference beam for every pixel. For the phase shift being proportional to the equivalent of dry mass the QPC data allow for the detection of cell growth, translocation as well as intracellular mass motion. In order to exploit the new option of observing cell reactions with CCHM we had to develop purpose fitted through-flow cultivation chamber that has to be available in identical twins (Fig. 1b). High metastatic A337/311RP (A3) cell [2] is imaged as hologram in Fig. 2a, which is then processed into standard quasi-3D phase presentation (Fig. 2b). In Fig. 2c the unwrapped phase image is shown, and series of these images served to elaboration of Dynamic Phase Differences (DPD) method [3] result of which is demonstrated in Fig. 2d. DPD in a structured way newly describes dynamics of cell motility. Results of DPD visualization of cell behaviour during cell cycle, under nutritional deprivation, and in cytotoxic stress will be shown both on videos of raw and processed data as well as in static DPD images describing the dynamic video information in only 2D (x,y) printable format [4].

References

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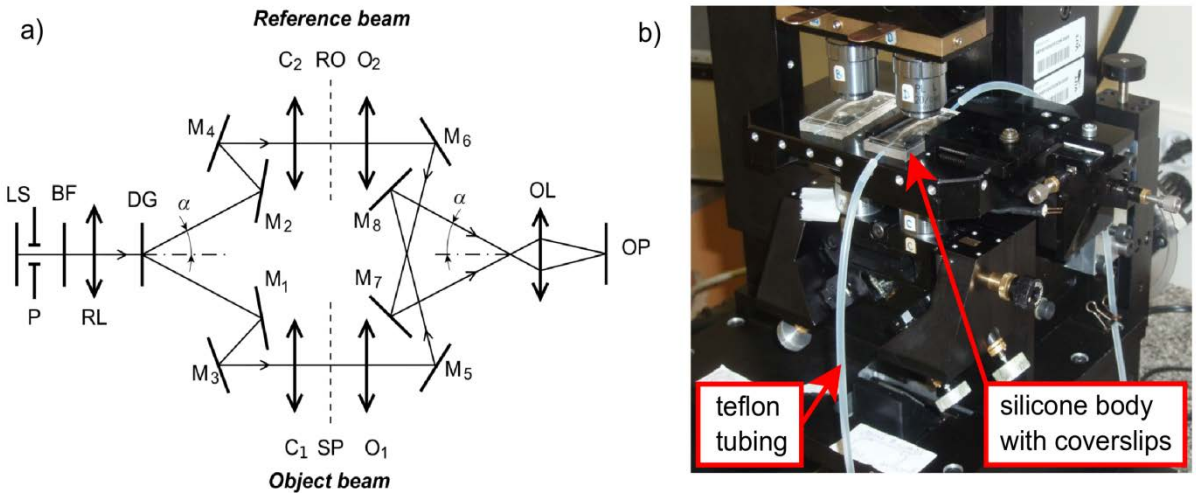

Figure 1. *a) Optical setup of CCHM: LS – light source, P – pinhole, BF – bandpass filter, RL – relay lens, DG – transmission diffraction grating, M – mirrors, C – condenser lenses, SP – specimen, RO – reference object, O – objectives, OL – output lens, OP – output plane, b) Assembly of perfusion chamber.*

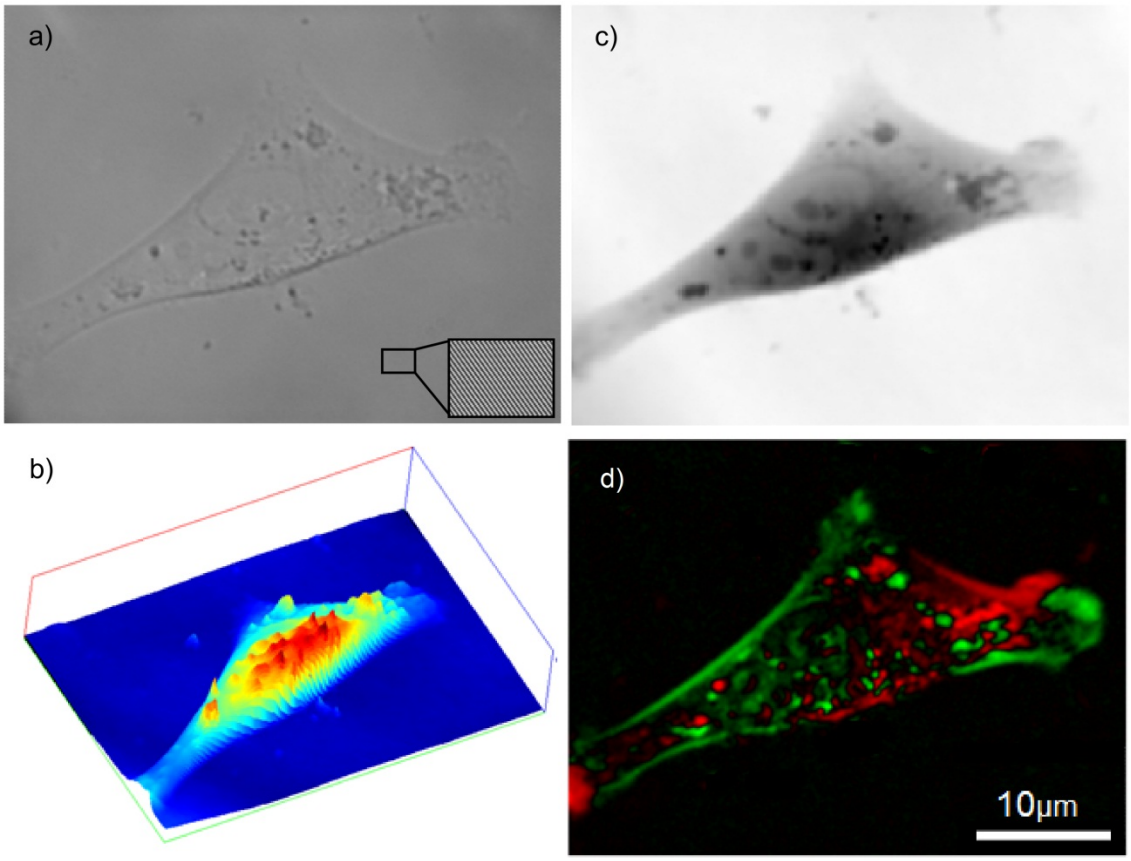

Figure 2. *Image processing: a) captured hologram of living cell, b) quasi-3D image phase, c) unwrapped image phase, d) example of DPD over 5 sec: red – increase of dry mass, green – decrease of dry mass.*