STRUCTURED ILLUMINATION BY A PINHOLE ARRAY FOR HOLOGRAPHIC MICROSCOPY

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The structured illumination of samples is known as one of the techniques for sub diffraction optical microscopy [1-3]. A spatial resolution beyond the optical diffraction limit is achievable by using nano light spots for scanning of the sample. We apply the structured illumination for digital inline holographic microscopy [4-6]. The structured illumination patterns are superposed by that of the sample. The interferogram can be detected directly by the CCD. Following results are discussed:

- Structured illumination is generated by interference using a pinhole array (micro hole and nano hole array). The interferences generate a set of light spots with a very high contrast. We present experimental results of a 4 x 4 micro hole array (hole diameter of 1.2 µm with distances of 15 µm) and of a 20 x 20 nano hole array (hole diameter of 350 nm with distances of 1 µm). Compared to a single pinhole illumination an enhancement of the maximum intensity by the factor 220 and more is measured.
- A new image reconstruction algorithm is developed. The object information is spread over the whole hologram. Therefore we separate the hologram into sub holograms, so called tiles. These tiles are separately processed very effectively [7].
- The structured illumination can be used to detect a set of holograms. The holograms are recorded using a low aperture angle to reduce aberrations, for example those caused by any glass sample carrier. The measurements with decreased aperture angle are combined to overcome their diffraction limit by creating and increasing a synthetic imaging aperture.
  Examples of wide field imaging of PMMA beads as well as of cells are given.

[1] G.L.Gustafsson, et.al., "Three- dimensional Resolution Doubling in Widefield Fluorescence Microscopy by structured Illumination," *Biophys JBioFAST*, (2008).
[2] M. H. Chowdhury et.al., "Imaging three-dimensional light propagation through periodic nanohole arrays using scanning aperture microscopy," *Applied Physics Letters*, **91,** 10, p. 103-118 (2007).
[3] L. Schermelleh, et.al. "Subdiffraction Multicolor Imaging of the Nuclear Periphery with 3D Structured Illumination," *Science,* **320,** pp.1332 (2008).
[4] D. Gabor, "A new microscopic principle," *Nature*, **161,** 777-778 (1948).
[5] R. Riesenberg, et.al., "Coherent light microscopy with a multi-spot source," *Proc. SPIE,* **6630**, pp. 66300I (2007).
[6] R. Riesenberg, et.al., "Synthetic Aperture Microscopy," *Program and Abstract Book*, FOM 2008, 88, (2008).
[7] M. Kanka et.al, "Reconstruction of high resolution holographic microscopic images", *Optics Letters*, accepted (2009).