SELF-CALIBRATING LENSLESS HOLOGRAPHIC MICROSCOPY FOR BLOOD CELL COUNTING

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A self-calibration technique for lensless compact chip-microscopes based on inline holography with pinhole illumination is presented. The pinhole illumination wave acts as reference and is needed for the reconstruction process. This reference wave is assumed to be spherical, so that its phase is already determined by the position of the pinhole in relation to the image sensor. It is shown that the reconstructed spatial resolution is strongly dependent on the estimation of the pinhole to sensor distance. A precision in the range of tens of microns was reached with a spatial resolution in the range of one micron. Therefore additional reference structures are prepared lithographically on the sample holder. The hologram, which contains the optical information about the sample as well as the reference marks, is used for calibration and image reconstruction at the same time. The spatial resolution achieved by the presented technique corresponds to the theoretical limit for the given numerical aperture of 0.66. The technique was applied to image blood smear samples.

Figure: Left above: Scheme of inline holographic microscope with additional crosses as marks (A, B) for self calibration. Left below: Blood Cell Counter with micro fluidic chip using a 3D inline holographic microscopic imaging. Right: Reconstructed human red blood cells (C, D) on a sample holder with cross marks as reference structures. The right reference wave as well as the right imaging relation is found by iteration with the known distance of 200 µm of the crosses A, B.