Pinhole-array and lensless micro-imaging with interferograms

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Gabors inline holography with a pinhole is extended by using a pinhole-array and a CCD. The arrangement is focused on microscopic imaging. The spatial resolution is given by the diameter of the pinholes and the dimensions of the CCD. By using a pinhole-array and a CCD with more than 1.3 Mpixels the field of view can be extended without lost of resolution. An example for dimensions and results of E. Coli imaging are given.

1 Digital inline holography

Fig. 1 shows the arrangement of Gabors inline holography for microscopy [1], [2]. Modern arrangements use CCDs for image registration, this technique is called digital inline holography [3].

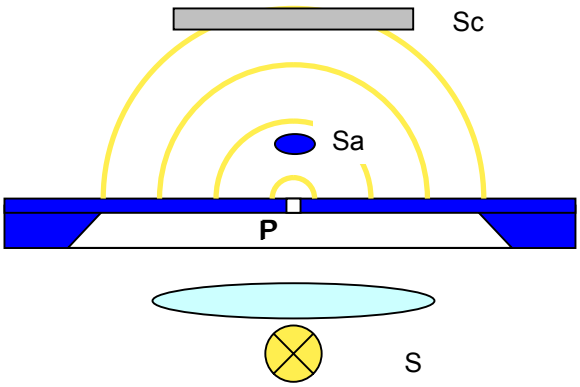

Fig. 1 *GABOR´s inline holography: S-coherent illumination, P-pinhole, Sc-Screen, Sa-sample*

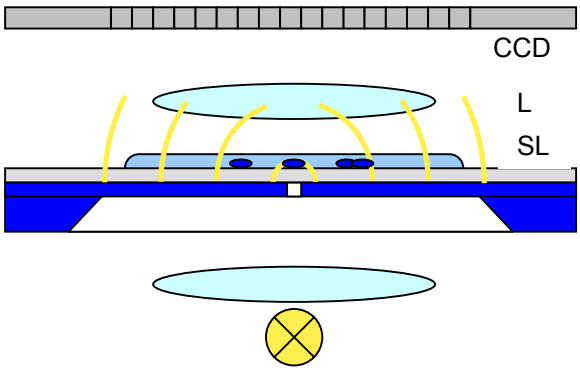

Fig. 2 *Digital inline holography with coherent light source, pinhole, CCD as a screen, SL-spacerlayer, which is carrying the sample, and samples in water, L-lens for comparing performance with that of a microscopic image.*

A part of the detected light is influenced by the sample, another part acts as a reference wave. So the area of the sample in the middle of the detected light cone (fig. 4) must be limited to about a half of the illuminating cone in one dimension. Gabors arrangement is used for a lateral resolution up to the diffraction limit like that of an optical microscope /2/. The set up shown in fig. 2 uses a lens to compare the image of the reconstructed interferograms with that of a microscope, results see fig. 5. In case of maximum spatial resolution a CCD with about 1.3 Mpixels is needed for detection of the field of view of a optical microscope. Today CCDs with more pixels are available. So the idea of holographic micro-imaging, a compact lensless microscope, with an extended field of view was born.

2 Pinhole-array and interferometric imaging

Fig. 3 shows the arrangement extended by a pinhole array. A spacerlayer carries the samples E. Coli in water. For good detection the interferogram on the CCD should include the first diffraction minima. The angle α, fig. 4, of illumination is determined by the pinhole diameter (d = 1 µm, α = 51 degrees, λ = 633 nm). A CCD with 1140 pixels in one dimension and a pixel-pitch of 3.35 µm is comparable with that of an optical microscope. The

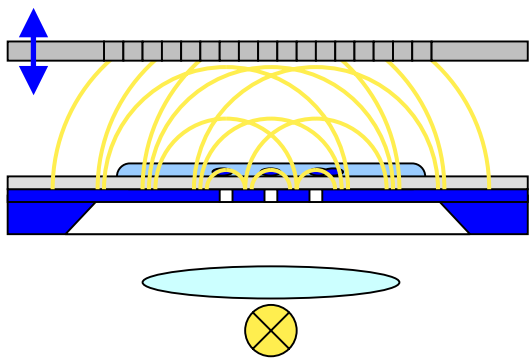

Fig. 3 *Imaging with interferograms with a pinhole-array and an extended field of view for more samples*

distance of the CCD to the pinhole-plane can be derived (c = 1600 µm). The sample illuminated by the pinhole generates additional interferences on the CCD. The detectable spatial frequency of interferograms is limited by the pixel size. The sample points with largest distances generate the highest interference-frequencies. The extension of the sample is limited by conditions of inline holography, too. So the distance b of the sample to the pinhole plane is derived (115 µm).

Much more extended CCDs are available, for example CCDs with 2280 pixels instead of 1140 in one dimension. If a second pinhole is applied, the distance to the first should be given by the condition of full illumination of a larger sample. For the example the distance between pinholes should be x < 70 µm. The sample size could be more than doubled and an additional part of the CCD is used. By this way an array of n = 54 pinholes would be useable ((1140 x 3.35 µm) / 70 µm) and the field of view could be extended by the factor n. The field of view of the state-of-the-art lens (50x/NA =0.7) of a microscope is 0.44 mm x 0.44 mm.

Limits are given by detection and by reconstruction, too. In the experiment the light of the pinhole-array is coherent. So all sample points can interfere with each other. An extended sample generates very high spatial frequencies on the CCD, which can not be detected by the limited size of pixels. The detectable interferograms limit the size of samples, but interferograms of n samples are superposed. For reconstruction a sequence of interferograms is generated. The techniques for reconstruction are described in [4], [5]. These techniques do not need any reference wave. Reconstruction in fig. 5 is done by a part of extented interferograms.

Further conditions concern the relation of screen size and the distance of the screen. The screen size should be larger than the distance of the sample. The limit of spatial resolution is in the order of the pinhole diameter. The limit of the number of pinholes used is also given by the detectable signal as well as of contrast-differences in the signals generated by superposition. Conditions will be considered in a further publication.

3 Acknowledgement

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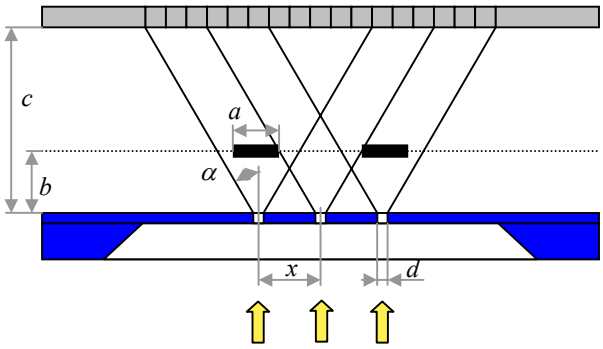

***Fig. 4**: Arrangement of a pinhole-array for lensless interferometric imaging to illustrate the extension of field of view (a - maximum sample size in case of using a single pinhole)*

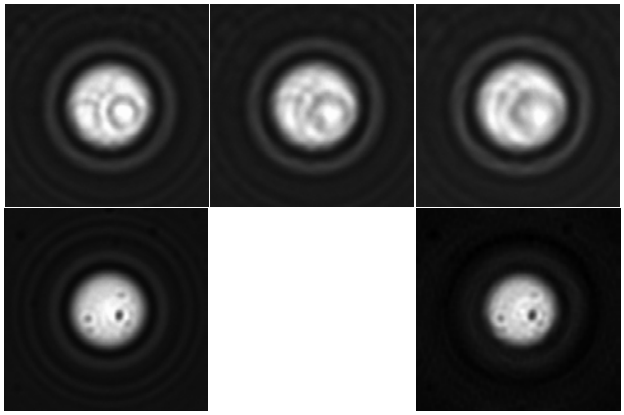

***Fig. 5**: First results are shown using two pinholes (diameter 1 µm, distance 4 µm, λ = 546 nm). As samples bacteria E. Coli are used on a spacer layer of 10 µm thickness.*
Upper row: Interferograms are shown (parts of the full interferogram) at a distance of 14 µm, 15 µm and 16 µm from the sample plane.
Below right: Reconstructed image /5/ with E. Coli (black and grey points with diameter of about 2 µm).
Below left: True microscopic image of the E. coli for comparison.

4 References

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