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## **Optimized Image Reconstruction in Digital Holography**

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We present the results of our research in digital in-line holography and discuss some challenges of the numerical reconstruction. We address the theoretical basics and describe an innovative reconstruction algorithm. Finally, we present some ideas for improving the reconstruction quality and propose a method for the estimation of global particle distribution.

The goal of our work is research and development of the digital holographic techniques for application in an experimental setup for observing the particles in a microfluidic canal. According to the method considered here the information about spatial particle positions can be obtained from a 2D image, called hologram, which is recorded by a CCD camera. All the necessary operations for image processing can then be carried out by the computer. The results of reconstruction processing can be represented in different ways, for example in a set of images which are corresponding to appropriate planes within the object volume at different z (depth) coordinates.

Our goal is to integrate a compact in-line holographic setup within an optical counter propagating tweezer system to observe the 3D flow of colloidal polystyrene particles at micron scale. Because of this small size an optical magnification is required. A telecentric telescope is applied due to its robustness with respect to longitudinal displacements.

The results presented in this research were obtained in a simplified installation without optical tweezers. The experimental setup consists of the telescope with pinhole producing the plane illumination wave and a confocal imaging system. A CCD camera is used for the recording (1280 x 1024 pixels,  $5.2 \times 5.2 \mu m^2$ ).

The imaging system forms the image of an absorbing object in a plane outside the sensor (Fig. 1). In this case the detector records the object's diffraction pattern named hologram.



Fig. 1 Hologram magnification by a telescope

The imaging system has a plane that is optically conjugated with the sensor plane. For simplicity we image the sensor backwards to the object volume. This model allows us to neglect the whole imaging system and is used by the developed reconstruction program which operates with the pixel size of 0.86µm. If we had a detector with pixel sizes of such small dimensions, the registered diffraction image would be restricted only by the sampling theorem. The actual imaging system has a spatial finite impulse response function (IRF) due to the limited extension of the pupil. Therefore, this spatial cut-off of the impulse response function is also introduced in the reconstruction algorithm.



**Fig. 2** The hologram (left) and the reconstruction (right) of Graphite corns on glass plate.

According to Babinet's principle the diffraction pattern generated by a small particle in the sensor plane (Fig. 2 left) can be described as the interference of the illuminating wave and the wave diffracted at a hole having the extension of the corresponding particle. In this case the diffracted wave has a negative sign or in other words its phase is shifted by  $\pi$ . This principle illustrates the modulation of the diffraction intensity. The extension of the diffraction order of the Fraunhofer diffraction pattern generated by the particle coincides with the extension of the first diffraction order of the Fraunhofer diffraction pattern generated by the corresponding hole.

One of the disadvantages of in-line holography is the twin image appearing around the object having the shape of rings after reconstruction (Fig. 2 right). The intensity of these rings and the size depend on the distance between object and recording plane. For large distances these marks become almost invisible. So there is a suitable depth range for registration of particles which allow the reconstruction of images with low contrast twin image noise.

The algorithm developed in this research project consists of two branches. One of them performs the image reconstruction eliminating the background noise modulation. In the second part of the program the appropriate impulse response function [2] is calculated numerically. The numerical reconstruction is based on the convolution algorithm. This operation simulates the propagation of the diffracted reference wave from the hologram plane to the reconstruction plane. In the reconstruction plane the images of the objects appear. After convolution the images go through non linear processing in order to be shown well graphically. Even if the background noise is eliminated the image background is not smooth so that the image of the object is not easily found. A non-linear intensity filtering can be carried out with different types of characteristic curves: The step-function results in a high-contrast black-white image. The function using a linear slope keeps the information of the object intensity, too. Finally, the whole 3D volume imaging (Fig. 3) is assembled from the obtained image stack in depth.



*Fig. 3* Experimental results. Reconstruction of the object volume 170x250x120µm<sup>3</sup>.

Some further filtering procedures may help to improve the image reconstruction. The recorded images include rather strong bias component caused by illumination. This reduces the contrast and the dynamic range of variable components containing all the information about particle location. This effect can be eliminated optically by applying a Fourier-filtering technique. If the imaging system generates a Fourier-plane (the rear objective's focal plane) it is possible to perform high-pass spatial filtering. In other words it is possible to selectively attenuate the parallel illumination beam by a dot-filter placed in the Fourier plane.

In the algorithm presented here the numerous convolutions are carried out in order to yield the images for different object volume planes. So this frame by frame processing takes a lot of time. The so called Parameterized Impulse Response Function (PIRF) combines section by section the whole depth range allowing only one convolution to obtain all the depth distribution information graphically shown according to scale [Fig. 4]. This method can be seen as a coherent wave front coding using astigmatism.



**Fig. 4** Reconstruction of the field 440x440µm<sup>2</sup> with parameterized impulse response function (left), depth scale (right)

A line analysis based on the Radon transformation offers a quantitative estimation of particle depth arrangement. It is evident that each line of the image corresponds to one point in the Radon domain. Therefore, we suppose that there are certain maxima in the Radon space which can bring the statistic information about depth of particles within the micro canal.

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