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Composite holograms in digital holographic microscopy

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A particle flow or randomly moving unicellular organisms within a microfluidic channel arranged in a classical in-line holographic setup is illuminated by coherent laser light and recorded by a CMOS Camera. The captured holographic video represents four-dimensional information with high complexity. We demonstrate some possibilities for the evaluation of the holographic video and the extraction of specific information about the particle flow.

1 Introduction

Digital holographic microscopy is a well established technique for the observation of three-dimensional objects. Very often, particle movements within small volumes need to be visualized, detected as well as measured. In conventional holography, this is only possible by capturing several holographic images on the photographic plate (multi-exposure holograms). The substitution of the photo plate by a digital solid state sensor array allows one to record a holographic video. The frames of this movie can be seen as a series of single snapshot holograms. Eventually, a four-dimensional process is recorded: three local coordinates *x,y,z* as well as the time coordinate *t*. In contrast to the large amount of information the user is generally interested in only few specific pieces of information of the recorded action. Therefore, some ideas of extraction are necessary. We demonstrate the application of filtering and projection. The amount of information is decreased by the filtering operation suppressing parts of the frequency spectrum. The projection reduces the coordinates, e.g. the dimensions.

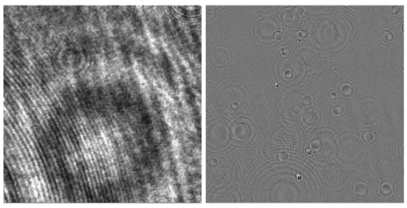

Fig. 1 *Example for a visualization of moving unicellular organisms derived from a holographic video (left) by subtracting of two consecutive holograms (right)*

2 Holographic Setup

A simple in-line holographic setup is used for the observation of a particle flow and the arbitrary travelling unicellular organisms within a microfluidic channel (Fig. 2). This channel is illuminated by a plane coherent wave of laser light at a wavelength of 543.5 nm. One part of this light is scattered by the objects. The whole light travels through the telecentric magnifying optical system composed of lenses L3, L4, interferes and eventually, impinges on the sensor array where the hologram is captured.

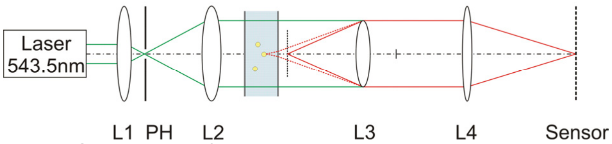

Fig. 2 *Schematic of the in-line holographic setup*

3 Reconstruction process: concept of filtering of a single shot hologram

The second step in holography is the reconstruction. In digital holography, this is done by digital processing based on the Kirchhoff Integral containing the Rayleigh-Sommerfeld diffraction term which directly expresses spherical waves:

$$E_{Rek}(x,y,z)=\int\limits_{-\infty}^{+\infty}\int\limits_{-\infty}^{+\infty}\sqrt{I_{Hol}(x_0,y_0)}\,\cos\sigma \times\frac{1}{\mathrm{j}\lambda}\,\frac{\exp\left[\,\mathrm{j}\frac{2\pi}{\lambda}\sqrt{z^2+(x-x_0)^2+(y-y_0)^2}\,\right]}{\sqrt{z^2+(x-x_0)^2+(y-y_0)^2}}\,\mathrm{d}x_0\,\mathrm{d}y_0 \tag{1}$$

Obviously, this expanded integral represents a convolution operation. In order to reduce the efforts on computational power and time, this processing is performed by using the Fourier Transformation, e.g. the Fast Fourier Transformation Algorithm. This offers the opportunity to add another processing, e.g. filtering, in spectral range realised by a transfer function $G(\nu_x,\nu_y)$:

$$\begin{aligned}&\Im^{-1}\left\{\Im\left\{\sqrt{I_{Hol}(x_0,y_0)}\right\}\cdot\Im\{g(x,y;z)\}\right\}\\ \mapsto\ &\Im^{-1}\left\{\Im\left\{\sqrt{I_{Hol}(x_0,y_0)}\right\}\cdot G(\nu_x,\nu_y)\cdot\Im\{g(x,y;z)\}\right\}\end{aligned} \tag{2}$$

This way, objects are easier observable because of the suppressed background inhomogeneities filtered out by a Gaussian high pass. Another possibility of extraction is the parameterisation of the impulse response function. In this case the parameterized impulse response function (PIRF) changes the phase term azimutally starting at x axis with the distance z_1 to y axis corresponding to distance z_2. All particles within this distance range are partially focused and defocused. The orientation of defocusing depends on the particle distance. At least, one reconstruction image is created containing the depth information of each particle [2]. This way, the z coordinate is reduced and the parameterisation can be seen as a projection along the depth direction.

4 Temporal projection of consecutive holographic images

Projection along the time axis is obtained by the generation of numerical multi-exposure holograms called composite hologram [3]. There are two principles possible: the addition and subtraction of temporally subsequent captured holograms. These combined holographic images can be reconstructed like common holograms. Eventually, the sequential addition of holograms allows the analysis of static components whereas the subtraction of consecutive snapshots is useful for the analysis of dynamic or time varying components. For instance, the reconstruction processing of a particle flow visualizes the instantaneous positions of the moving objects.

This composition offers two degrees of freedom: the number and the time base realised by overleaping holographic video frames. In the case of subtractive composite holograms the amount of combined holograms results in the number of imaged reconstructed objects (Fig. 3). By varying the frame overleap it is possible to adjust the range of measured particles velocities [4].

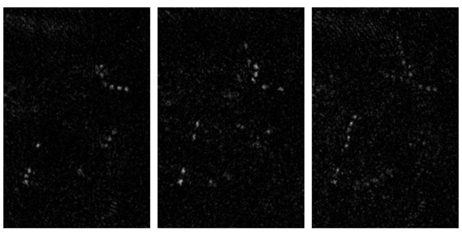

Fig. 3 *Comparison of reconstructed subtractive holograms of arbitrary travelling unicellular organisms: combination of 4 adjacent holograms (left), of 4 holograms skipping one frame (middle) and of 8 adjacent holograms (right)*

5 Higher organised composite holograms

Based on composite holograms there are higher organisations of subsequent holograms possible, the so called meta structures. Table 1 presents the opportunities of combinations as well as the emphasized information of these meta structures.

For instance, it is possible to generate additive holograms. After that, these additive composite holograms are subtractive combined to a meta hologram. This way, the background drift during a time period can be made visible. More interesting is the parameterisation of such temporally floating meta composite holograms.

Based on	additive Expansion	subtractive Expansion
additive	steady components	long time changes, drifts
subtractive	periodic processes*	dynamic deviations from periodic processes*

*requires an adopted observation time period

Tab. 1 *Survey over higher organized holograms*

The record of the standard deviation versus the time delivers the information of time windows where impacts (corresponding to peaks in Fig. 4) appeared to the microfluidic channel. Thus appropriate time intervals for observations with stable background can be determined very easily.

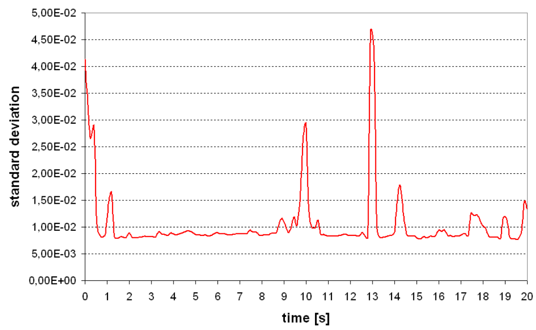

Fig. 4 *Standard deviation of a meta composite hologram vs. observation time*

6 Summary

Numerical processing of digital holographic videos enables additional opportunities for fast and easy investigation, studies, examination of micro-fluidic channel volumes.

High complexity of 4D information requires an information extraction realised by filtering and projection. The filtering operation can be easily integrated into the reconstruction algorithm. The projection can be performed locally (layers of focused particles) as well as temporally (concept of composite holograms).

Automatic evaluation of focused layers of moving and non-moving particles, impact-free time windows for observation is possible by creating structures of higher organisation called meta composite holograms.

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