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Particle detection in microfluidic systems

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In a microfluidic channel colloidal polystyrene spheres are manipulated by an optical tweezer. This contribution gives a short view over the 3D observation of moving and non-moving particles using a simple in-line hologram setup.

1 Introduction

The goal of our work is the observation of the 3D distribution of transparent objects (e.g. cells or silica spheres) within a fluidic channel. This is necessary in order e.g. to control the effect of the particle manipulation e.g. through counterpropagation optical tweezers. The conventional surveillance realised by a white light illumination is replaced by an in-line holographic imaging setup containing an optical system (telescope) for hologram magnification. It results in the advantage of numerical focussing and holographic image processing (3D rendering).

Moreover, the capture of a holographic video including the appropriate frame joining to a composite hologram [1] offers the possibility to dissect the analysis of moving and non-moving corpuscles.

2 Three-dimensional particle detection

2.1 Challenging particle detection

Tab. 1 lists a summary of special requirements for our experimental system.

particle characteristic	consequence
non-uniform particle shape and varying diameter	detection by simple correlation is not possible
diameter (1..3 microns) smaller than CCD pixel size	magnification is necessary
distribution of many particles within the whole volume	global survey/„reconstruction" is required
low intensity in comparison to the whole image	elimination of perturbations is needed

Tab. 1 *Particle characteristics and resulting consequences*

2.2 Hologram processing preparation: global filtering

The recorded hologram (Fig. 1 left) contains the diffraction fringes generated by the particle as well as illumination inhomogeneities, false light due to reflections as well as interference fringes caused by corpuscles outside the area of interest. A convenient way of eliminating these disturbances is to use a highpass filter suppressing all structures bigger than the maximum particle diameter (4 microns). In addition, the twin image is reduced [2]. This useful filtering is performed before the numerical reconstruction processing (Fig. 1 right).

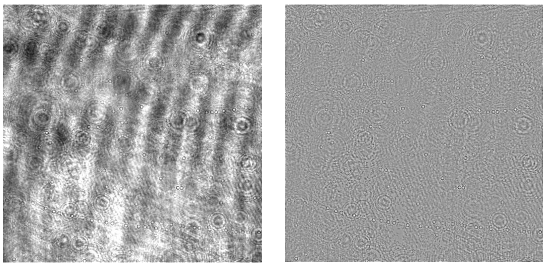

Fig. 1 *Recorded (left) and filtered (highpass) hologram (right)*

2.3 Global reconstruction

In general, an estimation of the ranges of particle grain existence can be realised by means of well known autofocus algorithms. At each normalised xy plane (along the depth) the sum, mean, standard deviation or gradient [3,4] is calculated. In the case of colloidal spheres the standard deviation results in a reliable information about the quality and quantity of particles. On every local maximum focussed particles are observable. For instance, we can detect the Bottom of the microchannel at a depth of 286µm (Fig. 2).

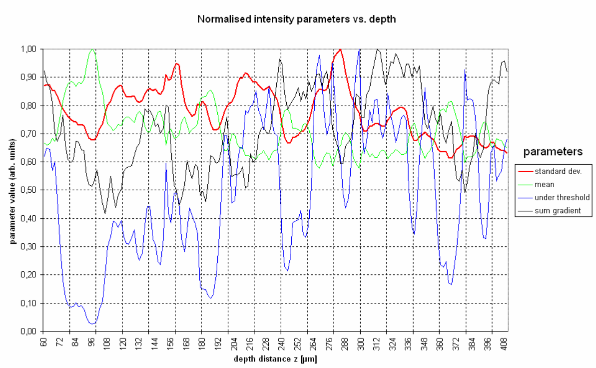

Fig. 2 *Parameters vs. reconstruction distance*

2.4 Local particle search

A simple and fast 3D minimum search algorithm is applied using a thresholded trace for local extrema along the z direction. Then, the same search is utilised along the y direction. Finally, both results are combined into a data list of coordinates x,y,z and strength.

3 From video to composite hologram

A particle flow video is captured at a rate of 15 frames per second. The topic "composite hologram" stands for numerical generation of a multi-exposure hologram. There are two ways for holographic examination:

Static analysis - exploration of local and time invariant corpuscles within the channel flow. This is performed by addition of subsequent holographic frames.

Dynamic analysis - inspection of moving particle flow. It can be performed by alternating addition/subtraction of successive holographic frames of even quantum [1].

Both methods for the numerical evaluation offer the following degrees of freedom at a given frame rate: total number of frames, number of skipped frames.

3.1 Static analysis

The simple addition of consecutive frames attenuates the variable parts of the images. Fig. 3 shows a comparison of the global parameter standard deviation by variation of the number of added and neglected frames for an estimation of the efficacy.

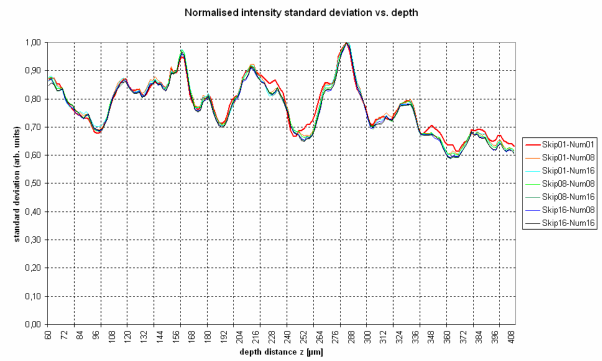

Fig. 3 *Standard deviations at frame counts vs. reconstructed depth. The strong red line stands for single captured hologram (static and dynamic particles), the weak lines for multiple combined frames.*

This method leads to a good suppression of moving components at small frame counts.

3.2 Dynamic analysis

This frame processing can be viewed as the interference of holograms with opposite phases. The static hologram information is being cancelled, while all other dynamically varying parts are appearing bright. Thus, only moving parts are becoming visible by eliminating the perturbing background and reducing noise [1]. The reconstruction processing visualises the instantaneous positions of the moving particles.

The overlay of holographic frames with alternating signs influences the visibility of closer grains. The particles outside along the trajectory appear brighter because of the superposition with only one "self originated" neighbour. This destructive interference results in a gap between these two unique particle images (Fig. 4, left picture - red marks).

Total number of frames. Variable external influences during the video capture (instable illumination, vibrations) as well as the compressed 8 bit quantisation result in a continuous increase of noise with an increasing number of combined frames.

Skipping a number of frames. The possibility to neglect a certain number of frames allows one to flexibly choose the timebase. By varation of the frame overleap it is possible to adjust the range of particle velocities which can be recorded. Very slow particles rolling on the bottom of the microchannel can thus be made visible (Fig. 4 - yellow marks).

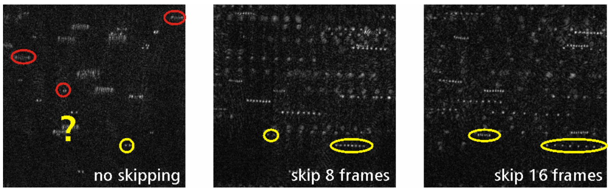

Fig. 4 *Time scaling by frame skipping, z = 286µm*

4 Summary

A simple digital in-line setup enables the observation of particles within a microfluidic channel. By means of composite holograms the 4D investigation (x,y,z,time) is reduced to a 3D study. In summary, a video block of 256 frames (17 seconds) was already sufficient to realise reliable static and dynamic examinations.

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