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An experimental investigation of three-dimensional particle aggregation using digital holographic microscopy

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ABSTRACT

The tendency of particles to aggregate depends on particle-particle and particle-fluid interactions. These interactions can be characterized but it requires accurate 3D measurements of particle distributions. We introduce the application of an off-axis digital holographic microscopy for measuring distributions of dense micrometer (2 μm) particles in a liquid solution. We demonstrate that digital holographic microscopy is capable of recording the instantaneous 3D position of particles in a flow volume. A new reconstruction method that aids identification of particle images was used in this work. About 62% of the expected number of particles within the interrogated flow volume was detected. Based on the 3D position of individual particles, the tendency of particle to aggregate is investigated. Results show that relatively few particles (around 5–10 of a cohort of 1500) were aggregates. This number did not change significantly with time.

1. Introduction

The tendency of platelets to clump together at sites of vascular injury, or commonly referred to as platelet aggregation, is important to stop continuous bleeding [1]. This is helped by the formation of thrombus or blood clot in a process known as thrombosis. However, thrombus can also be detrimental in thrombosis when the clots happen to partially or completely block flow of blood through healthy blood vessels, leading to the development of cardiovascular disease [2]. In addition, platelets aggregation is also influenced by the surrounding hemodynamic environments especially in the recirculation and stagnation flow regions and in stenosed micro-channels [3].

Meanwhile, use of therapeutic platelet substitute (e.g. HaemoPlax™) could be an option especially for leukaemic patients to overcome platelet deficiency as a result of undergoing chemotherapy. HaemoPlax™ is based on a proven technology in which a fibrinogen-binding peptide is linked to an insoluble carrier of human albumin through a polyethylene glycol spacer that spatially maximizes binding. On administration, the microsphere is coated in the patient's own inactive fibrinogen and remains inactive until it contacts thrombin, a naturally occurring protein at a site of injury. This contact activates the patient's platelets allowing them to bind to the fibrinogen on the HaemoPlax™ particles, providing an injury site targeted adjunct in patients with insufficient platelet activity. Aggregation prior to activation is clearly undesirable. It is noted that the tendency of particle aggregation depends on particle-particle and particle-fluid interactions. These interactions are closely influenced by the surrounding hemodynamic environments. HaemoPlax™ is stable as delivered, but it has been suggested that high shear rates observed during injection and within the bloodstream could affect coatings and promote aggregation of the particle. The main objective of this study is to experimentally investigate the tendency of therapeutic platelet substitute to aggregate under real blood conditions using digital holographic microscopy.

A new application of holography in the field of optical microscopy has introduced a new imaging technology, known as digital holographic microscopy [4]. Based on the principle of coherence imaging, it allows reconstruction of a three-dimensional (3D) volumetric field from a single hologram capture. The technique relies on digital image and numerical processings to obtain quantitative information in non-invasive and real-time conditions. The essence of holographic imaging lies in the fact that, when coherent light propagates through a semi-transparent object, its amplitude and phase get modulated due to light-matter interaction. This effectively means that the entire 3D structure of the object is coded in the form of scattered wavefronts which eventually incident on to imaging sensors.

Imaging of a relatively low particle concentration is a straightforward process. It however becomes difficult as the particle concentration increases due to noise contributed by out-of-focus particle images [5,6], where the noise severity increases proportional to depth volume [7]. The ability of any digital holographic microscope to detect a number of particles within a system has

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been shown dependent on several important parameters namely: (a) particle concentration, n_s; (b) particle diameter, d; and (c) axial depth, L. The overall relationship was first articulated by Royer who defined the shadow density, s_d as a means to quantitatively determine the image degradation of a particular hologram [7].

Additionally, the maximum particle concentration that can be reliably detected, however, depends on the accuracy of the particle image identification technique/algorithm to differentiate reliable images from noise [8]. For instance, Sheng et al. [6] demonstrated the application of in-line digital holographic microscope for 3D particle distributions. Using a segmentation for particle image identification [9], about 5679 particles and their 3D positions were identified. The segmentation method substitutes each identified particle area at different depths with a mean circular mask and eventually a cylindrical volume is effectively created representing the original reconstructed particle. As a consequence of the in-line setup, the particles suffer poor depth-of-focus where the reported depth-of-focus of 3 μm particle is approximately ten particle diameters. In the previous work of Wormald and Coupland [8], an off-axis digital holographic microscope was developed to spatially separate noise contributed by the undiffracted beam and virtual images. The particle identification was achieved by finding the brightest pixel in the reconstruction volume, masking a suitable particle image sized region from the reconstruction and repeating the process. To avoid measuring the same particle twice the mask was cylindrical and extended into many neighboring planes above and below the plane of best focus. Though this technique works well in sparsely seeded flow, it severely reduces the possibility of detecting out-of-focus particles that are directly above (or below) each other.

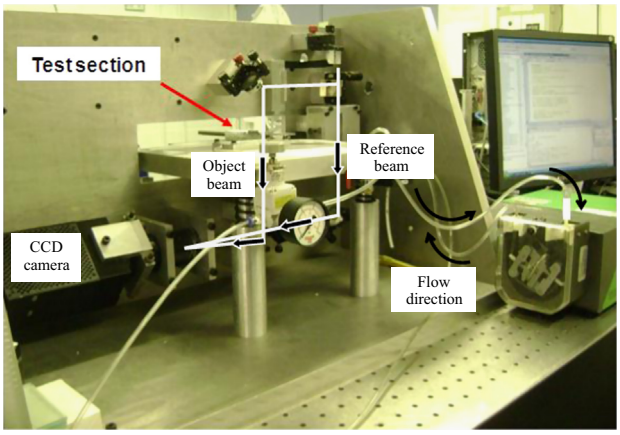

Fig. 1. Digital holograhic microscope used for the experiment [8]

The aforementioned issue of differentiating in-focus and out-of-focus particles can be overcome using Canny edge detection to segmentize particle image from the background, as proposed by Darakis et al. [10]. In this method, an appropriate threshold value of the intensity was first set enabling in-focus particles characterized by strong edges to be detected whilst highly out-of-focus particles without distinctive edges automatically left undetected. The 3D position was determined at the best focused depth, at the plane in which the calculated mean intensity and variance of the pixels surrounded by the edges were the lowest. Interestingly, the algorithm was shown applicable for measuring both spherical and needle-shaped particles in suspension. It is however, limited to the measurement of non- spherical particles in 2D projection as viewed from the camera. Kempkes et al. [11] developed a different algorithm which capable of characterizing needle-shaped particles randomly distributed in a 3D volume without a *priori* knowledge. It is also based on (particle image segmentation) converting the in-focus images of the particle at different depths into binary images which can be later seen as a cumulative 3D point cloud. A 3D line fitting using principal component analysis was used to estimate 3D position, length and orientation of the particle, but at the expense of high computational load. By superimposing the 2D projection [10] and the 3D point cloud [11] algorithms, Khanam et al. [12,13] eventually managed to measure the 3D real characteristics of the particles originally restrained by the former algorithm at a significantly reduced computational load of the latter algorithm.

In this study, a new particle image identification technique was developed to maximize the number of detected particle images in a real experimental flow using an off-axis digital holographic microscope. Because this study is concerned with aggregation of therapeutic platelet substitute (approximately 2 μm in diameter), the statistics of particles that are in close proximity is of great concern. It is noted that the state of particle aggregation also relies on the number of particles that can reliably be detected. Based on the individual 3D positions of each particle, the state of aggregation concerning therapeutic platelet substitute is finally determined. This study is foreseen to be vital in the field of hemodynamic research as well as in biomedical applications. The experiment is described in the following section.

2. Digital holographic microscopy

2.1. Digital holographic recording

The holographic microscope used for this study is shown in Fig. 1 and Fig. 2a. The microscope operates in transmission at a wavelength

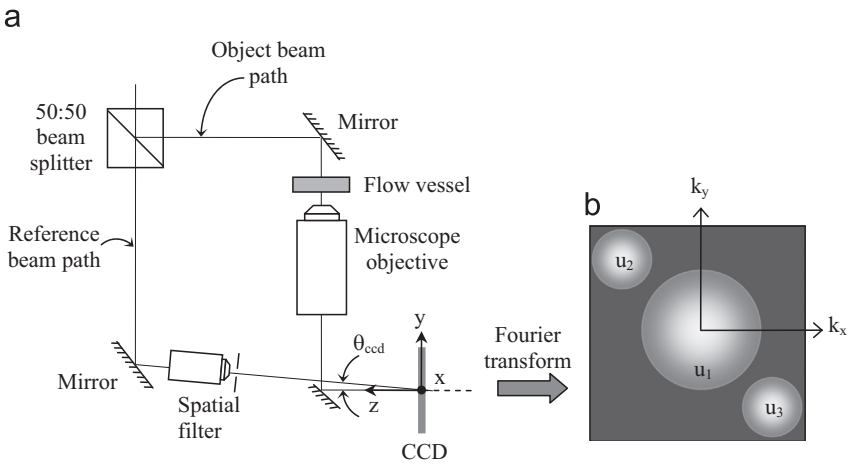

Fig. 2. (a) Optical geometry of a digital off-axis forward-scatter holographic microscope, and (b) Fourier transform of the interference pattern recorded on the CCD plane.