Density-Based Separation of Microparticles Using Magnetic Levitation Technology Integrated on Lensless Holographic Microscopy Platform

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***Abstract*—Microparticle/cell separation is one of the most important applications in the field of biomedical sciences particularly for cell sorting and protein assays. There are variety of different separation technologies introduced in the literature that the main limitations are large amount of sample, expensive chemical use besides of requirement of a labeling procedure (i.e. fluorescent/magnetic labeling), complex machinery, and high operational costs. Magnetic levitation-based separation offers simple, rapid and precise separation of microparticles based on their densities by suspending them in a glass microcapillary between two opposing magnets. Traditionally, magnetic levitation-based microparticle separation and identification procedure is performed by imaging under bulky microscopes composed of fragile and expensive optics and require trained personnel to operate which makes the whole procedure costly, time consuming and prone to human error. Lensless digital inline holographic microscope (LDIHM) eliminates the need for sophisticated optics by replacing simple illumination and recording scheme that can be reduced into few widely-available and cost-effective components. Thus, inspection procedure is mostly carried out on digitally processing captured holograms so that dependency on optical components and human error is dramatically reduced alongside using cost-effective and handheld device. Here, we introduce a novel hybrid platform that brings the advantages of magnetic levitation system with lensless digital inline holographic microscope for precise separation and identification of microparticles based on their densities. In the platform, it was shown that 1.026 g/mL and 1.090 g/mL microparticles were successfully identified.**

Keywords—microparticle separation, magnetic levitation, lensless digital inline holographic microscopy, density measurement

I. INTRODUCTION

Separation of microparticles/cells has been the interest of many fields regarding diagnostics, chemical analyses, food and environmental investigations, etc. [1]. However, traditional separation methods suffer from complex instrumentation setup, high amounts of sample and expensive reagents [2]. In these methods, separation of microparticles is achieved using fluorescent or magnetic labels. However, labeling step makes the separation procedure labor intensive, time-consuming and costly [3]. On the other hand, label-free separation of microparticles based on physical characteristics is a promising tool. Lately, magnetic levitation technology has become an exciting approach for separation of microparticles based on their densities [4]. Magnetic levitation is a magnetophoretic phenomenon. A particle suspended in a paramagnetic medium levitates at an equilibrium position where the magnetic force and buoyancy force acting on the particle balance each other. Standard configuration of magnetic levitation platform consists of two magnets with same poles face each other that creates a non-uniform magnetic field between the magnets [5]. A particle levitates at a distinct levitation height determined by the magnetic susceptibility and density difference between the particle and the surrounding medium. Diamagnetic particles are pushed towards the field of magnetic field minima, corresponding to the centerline between the two magnets. The relative density of a diamagnetic particle compared to the surrounding medium determines the levitation position of the particle. The particle levitates above the centerline if the density of the medium is higher than the density of the particle. Yet, if the particle is denser than the medium, it levitates below the centerline. Magnetic levitation has been exploited in separation of diverse particles including cell populations [6-9], materials [10, 11], and food samples [12] due its miniaturized design [6], contactless nature on biological entities [13, 14], low cost, and high accuracy [2]. In a magnetic levitation platform, microparticles can be identified by inspecting their levitation heights under a traditional microscope.

Traditionally, microscopes are gold standard instruments for examining microparticles. However, microscopes are generally heavy and bulky instruments that are composed of highly precise, fragile optics which make the microscopes expensive for a more practical application such as point-of-care testing. Additionally, microscopes are required to be operated by a trained laboratory technician. Fine adjustment is necessary to obtain high quality images of the sample. Hence, image quality of the sample can be changed with respect to the configuration of the microscope and the level of expertise of the technician.

Lensless digital inline holographic microscopes (LDIHMs) have been attractive for the last decade due to their simplicity, portability and capability of providing high

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resolution, high field of view and quantitative images. In LDIHM, the use of highly fragile and high cost optics is eliminated by replacing them with a relatively simple illumination and recording scheme. Illumination system can be reduced into a simple light emitting diode (LED) coupled with low aperture size (~100 µm) pinhole. The recording system is only composed of an imaging sensor array without introducing any lens system for focusing purposes. LED is spatially filtered by a low aperture size pinhole to obtain quasi-monochromatic light source. After filtering the LED light, sample is directly placed just at the top of the imaging sensor array to record the interference pattern (hologram) between the light diffracted from the object (object wave) and the background light (reference wave). Therefore, entire active area of the imaging sensor can be used directly to achieve large field of view (>10 mm^2), which is 50 times greater than that of a conventional microscope equipped with 40× objective. Spatial resolution of 1.55 µm can be easily obtained in LDIHM, which is good enough for imaging few micron-sized particles [15].

After recording the hologram of the sample, it is back-propagated from the sensor plane to object plane by using a spatial transfer function. The exact object plane is found by scanning the propagated hologram along z-axis at different steps (e.g. 100 µm). Thus, instead of manually scanning to find the focused real object images, an auto-focus algorithm can be deployed [16]. As a result, human expertise is minimally needed, and the dependency of microscope configuration is dramatically reduced.

In this study, we present a new hybrid platform for separating microparticles based on their densities using magnetic levitation technology. Levitation heights of microparticles are inspected using LDIHM coupled to the platform to identify microparticles with different densities. This system introduces a low-cost, compact and portable design by combining magnetic levitation and LDIHM technologies to separate and identify microparticles having different densities. As a result, unlike other imaging techniques, LDIHM is capable of eliminating sophisticated optics, and it also enables digital processing and handheld measurements that are particularly useful for magnetic levitation technology depending on bulky and costly traditional bench-top light microscopes in order to visualize the sample within the microcapillary channel. In the future, capability of the platform can be extended into screening of cells with minute density differences and sorting them for further analysis.

II. Experimental

A. Design of the platform

Magnetic levitation system consists of two magnets (N52 grade neodymium magnets, 50 mm length × 2 mm width × 5 mm height) inserted as opposing configurations (same poles face each other) and a glass microcapillary channel placed between the magnets (50 mm length × 1 mm width × 1 mm height) in which a sample solution is loaded.

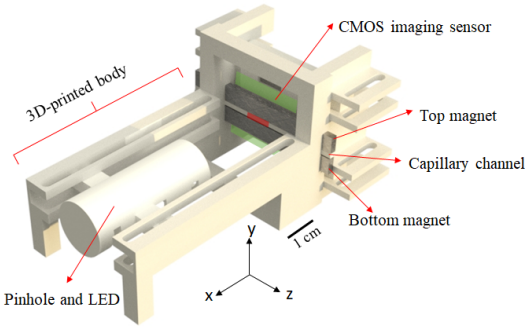

Fig. 1. Illustration of the hybrid platform composed of a magnetic levitation system and LDIHM.

LDIHM is composed of illumination and recording structures. Illumination is achieved by a 650 nm LED in wavelength filtered by a pinhole with diameter of 100 µm. The distance between pinhole to glass capillary is less than 5 cm. Holograms are captured with 8-megapixel Sony IMX219 complementary metal oxide semiconductor (CMOS) imaging sensor whose pixel size is 1.12 µm with 3280 (H) x 2464 (W) active pixels. CMOS imaging sensor is placed beneath of a glass microcapillary between two opposing magnets with close proximity (< 1 mm). Microcapillary is illuminated by LED coupled with pinhole, and the resulting interference pattern which is called hologram between constant phase background light (reference wave) and the light diffracted from microparticles within the capillary (object wave) are recorded by using CMOS imaging sensor.

B. Sample Preparation

Diamagnetic polyethylene standard beads with a density of 1.026 g/mL (10-20 µm in size) and 1.090 g/mL (20-27 µm in size) (Cospheric, LLC) were levitated in the magnetic levitation platform. In this regard, they are suspended in phosphate buffer saline (PBS) (Gibco^{TM}) at pH 7.4 with 1% (w/v) of Pluronic® F-127. Then, the solution is mixed with paramagnetic medium, Gd^{+3} (Gadobutrol ®), to achieve a 30 mM of Gd^{+3}-based levitation medium. 40 µL of the resulting solution is loaded into the microcapillary channel.

The beads levitated in the paramagnetic medium with a 1.030 g/mL density for 10 min were analyzed in terms of levitation height (i.e., the distance of the beads from the surface of the bottom magnet).

C. Magnetic Levitation Principle

Two magnets are placed in the platform as the same magnetic poles facing each other. This configuration creates a magnetic field gradient where the middle of the magnets corresponds to magnetic field minima. As shown in Fig. 2, a randomly distributed particle inside the paramagnetic medium levitates at an equilibrium height where the magnetic force (F_M) is equal to buoyancy force (F_B).

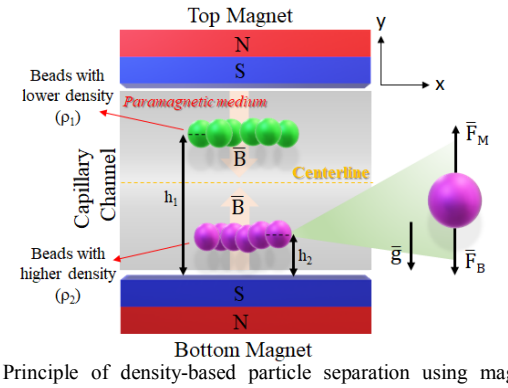

Fig. 2. Principle of density-based particle separation using magnetic levitation. The particle having a higher density levitates at lower height compared to a lighter particle ($\rho_1<\rho_2$ and $h_1>h_2$). Hence, particles can be distinguished by their unique levitation height.

Levitation height of a particle depends on magnetic susceptibility (χ_P-χ_M) and density difference (ρ_P-ρ_M) between the particle and the surrounding medium. This levitation height can be calculated as in Eq. 1.

$$\frac{V.(\chi_p-\chi_m)}{\mu_0}(\vec{B}.\nabla)\vec{B} = V(\rho_P-\rho_M)g \qquad (1)$$

The left-hand side of Eq. 1 represents the F_M acting on the particle where V is the volume of the particle, χ_p and χ_m are the magnetic susceptibilities of particle and surrounding medium (unitless), respectively. μ_0 is the permeability of free space (1.2566×10^{-6} kg.m.A^{-2}.s^{-2}), B is the magnetic flux density (Tesla, T), and ∇ is the del operator. The right-hand side of Eq. 1 represents the F_B, where (g) is the gravitational acceleration (9.8 m.s^{-2}), ρ_P and ρ_M are volumetric densities of particle and the medium.

D. Back-Propagation of Holograms and Measuring Levitation Heights of Microparticles

Images are digitally processed through custom developed MATLAB script. Captured holograms by CMOS imaging sensor are digitally back-propagated to retrieve the real image along the z-axis. Holograms are converted into real images by applying Angular Spectrum Method as follows [15]:

$$\Psi_P(x,y;z) = \mathcal{F}^{-1}\{\mathcal{F}\{\Psi_{Po}(x,y)\}H(k_x,k_y;z)\} \qquad (2)$$

where $\mathcal{F}$ is Fourier transform and $H(k_x,k_y;z)$ is the spatial transfer function:

$$H(p,q) = exp\left[-jk_oz\sqrt{1-\frac{(p\Delta_{k_x})^2}{k_o^2}-\frac{(q\Delta_{k_y})^2}{k_o^2}}\right] \qquad (3)$$

The wave number is $k_o = w_o/v$, where w_o and v are angular frequency (rad/s) and the speed of the wave front, respectively. (x,y) and (p,q) are indices of the sampled imaged in both spatial and Fourier domain. Frequency resolutions (in radian per unit of length) are $\Delta_{k_x}= 2\pi/M\Delta_x$ and $\Delta_{k_y}= 2\pi/N\Delta_y$ where Δ_x and Δ_y are sampling periods, and the number of samples in the direction of x and y are indicated as M and N, respectively. The distance between the sample and imaging sensor, z, is obtained through propagating the hologram from detector to object plane. Since reconstructed image $\Psi_P(x,y;z))$ is complex-valued at the distance z where the object is, it is separated into real and imaginary images. Moreover, amplitude and phase images are obtained through calculating the modulus and argument of complex-valued reconstructed image ($\Psi_P(x,y;z)$), respectively.

Once the in-focus real image is achieved by propagating the hologram to object plane (Eq. 2), evaluation of levitation heights of microparticles is performed by digitally processing the image as shown in Fig. 3 [17]. Firstly, hologram is captured (Fig. 4a) and reconstructed into the amplitude image that is processed for further analysis (Fig. 4b). Contrast of the reconstructed image is enhanced for identifying and segmenting the microparticles in the microcapillary channel. Hough transformation is then utilized for precisely calculating the shift angle caused by positioning of CMOS imaging sensor with respect to the bottom magnet. Reconstructed image is rotated based on the angle calculated with Hough transform to align the side of the magnet to the x-axis (Fig. 4c). Secondly, image is segmented by thresholding for local minima of the microparticles' pixel values to obtain the binary mask. Due to the presence of twin image in reconstructed holographic images (Fig. 4c), it can be challenging to obtain a smooth binary mask from the polluted reconstruction image. Thus, simply applying a global thresholding procedure for obtaining binary mask of the microparticles will not give satisfactory results and may potentially lead to miss many of the microparticles. Hence, Sauvola local image thresholding is used after fine-tuning of parameters regarding neighborhood size and sensitivity of thresholding (Fig. 4d). Sauvola method performs quite well for reconstructed holographic images over traditional segmentation algorithms, because the background of reconstructed image is not uniform that twin image causes irregular intensity variations along the image mentioned in detail above. So, Sauvola algorithm applies local thresholding over the entire image instead of applying a single global threshold for every pixel that mean and standard deviation of local neighborhood relations are taken into account. Further morphological operations (e.g. closing) can be applied to eliminate the irrelevant pixels and preventing miscalculations for evaluating height values. Lastly, blobs in the binary mask are detected by identifying the labels of connected regions. Afterwards, levitation height of each region (microparticle) is calculated as measuring the vertical distances between each segmented microparticles' center of gravity to a fixed reference plane (e.g. edge of the bottom magnet) (Fig. 4e).

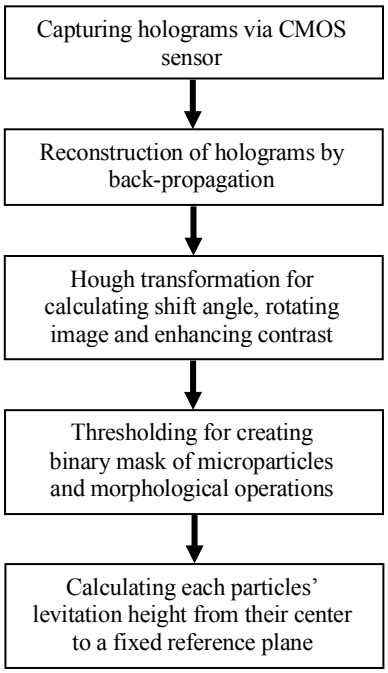

Fig. 3. End-to-end flow diagram of measuring densities of microparticles within capillary channel through processing captured holograms

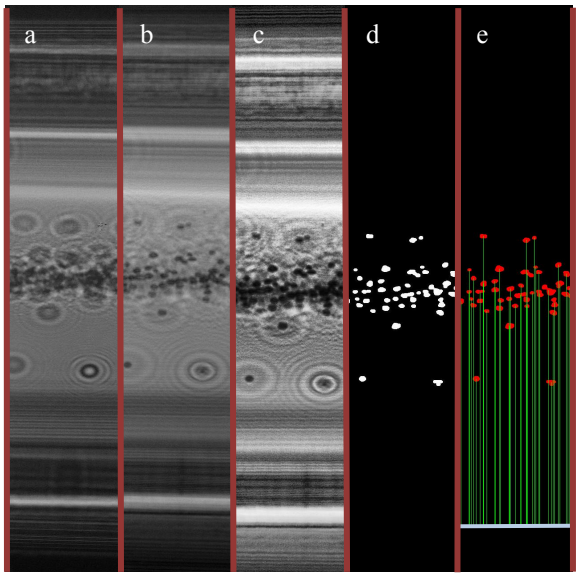

Fig. 4. Illustration of digital image processing steps described in Fig. 3 on a small section of captured image of 1.026 g/mL microparticles. (a) Captured hologram, (b) amplitude image of reconstructed hologram, (c) resulted image after Hough transformation, rotation and contrast enhancement, (d) resulted image after thresholding for binary mask creation and morphological operations to eliminate irrelevant noise, (e) corresponding levitation heights of microparticles with respect to the bottom magnet (blue horizontal line).

III. Results And Discussion

In this work, magnetic levitation-based separation was applied for polyethylene microparticles with two different densities (i.e., 1.026 g/mL and 1.090 g/mL). Since the microparticles are diamagnetic in nature, they are pushed away from the larger magnetic field site to the lower and remain stationary when magnetic and buoyancy forces are equal. As shown in Fig. 5, the microparticles with higher density (i.e. 1.090 g/mL) levitated closer to the bottom of the magnet compared to the microparticles with lower density (i.e. 1.026 g/mL) when the beads reached a stationary levitation position. The average levitation position of higher density beads is 158 μm below the centerline whereas the lower density beads levitated 82 μm above the centerline. Since the density of the paramagnetic medium is 1.030 g/mL, it was expected to levitate beads with 1.026 g/mL density above the centerline and beads with 1.090 g/mL density below the centerline. The levitation heights of beads were measured (Fig. 6) and statistically analyzed according to a t-test (GraphPad Version 6.0). The results revealed that the levitation heights of two standard density-microparticles are significantly different with a P value < 0.0001. Therefore, the proposed platform is capable of identifying beads with different densities by measuring only levitation height and could be used to identify cells or particles of different densities by levitating them until a stable levitation position is reached using only minute amounts of sample (40 μL) and short analysis time (10 min) per test.

Furthermore, our study introduces a compact, handheld and robust microparticle separation and identification platform based on magnetic levitation principle. As imaging system of the platform is not composed of fragile and sophisticated optical components such as lenses and mirrors, the platform is insensitive to environmental impacts. It is also cost-effective and requires low maintenance as well. Finally, microparticle identification procedure is conducted automatically on the platform so that method eliminates possible user errors. This platform will be the basis of a new type of a portable cytometer device to be used for sorting of heterogenous cell populations, rare cell identification, also many other applications regarding precise-monitoring of microparticle-based assays [18].

IV. Conclusion

Density is known to be an important physical property especially for separating microparticles/cells within a heterogeneous solution and studying cell assays in vitro. Proposed platform offers low-cost, portable and rapid separation and identification of microparticles based on their densities in a minute amount of sample. This new platform utilizes the advantages of both magnetic levitation and lensless digital inline holographic microscopy to precisely monitor levitation heights of microparticles, and that is used for separating and identifying microparticles automatically. The platform relies on digital processing of holograms. Hence, imaging of microparticles would be insensitive to operator errors. The platform is also composed of low-cost elements (< $100) assembled together with 3D-printed parts. Therefore, the dissemination of the

platform would be easy. Future studies will be focused on identifying different types of cells that could potentially be used for point-of-care diagnostics and prognostics applications.

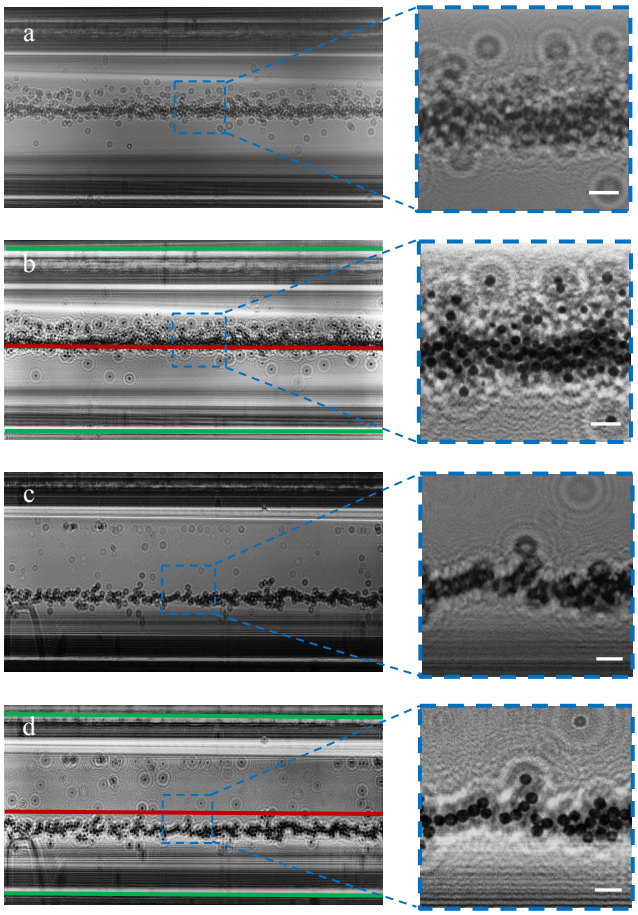

Fig. 5. Imaging of microparticles in the platform. (a) and (c) are holograms, (b) and (d) are reconstruction of the holograms of microparticles with 1.026 and 1.090 g/mL densities, respectively. Scale bar is 50 µm. Green and red lines indicate the edges and centerline of the magnets, respectively.

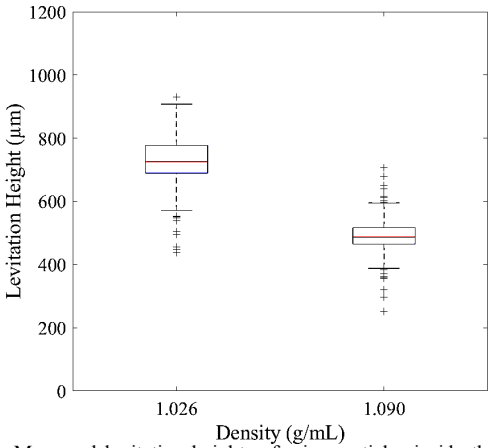

Fig. 6. Measured levitation heights of microparticles inside the platform using 30 mM paramagnetic medium.

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