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Short communication

Magnetic immunoassay platform based on the planar frequency mixing magnetic technique

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ABSTRACT

We represent the experimental results of our planar-frequency mixing magnetic detection (p-FMMD) technique to obtain 2D superparamagnetic images for magnetic immunoassay purpose. The imaging of magnetic beads is based on the nonlinear magnetic characteristics inherent in superparamagnetic materials. The p-FMMD records the sum-frequency components originating from both a high and a low frequency magnetic field incident on the magnetically nonlinear nanoparticles. In this study, we apply the p-FMMD technique to 2D scanning imaging of superparamagnetic iron oxide nanoparticles (SPIONs) in a microfluidic platform. Our p-FMMD system enables to acquire planar images of SPIONs filled in a microchannel as narrow as 30 μm in width. The minimum detectable amount is $\sim 1.0 \times 10^8$ beads of 100 nm size. The system shows a spatial resolution enabling to distinguish between two distinct channels even 2 mm apart from each other. Our p-FMMD system as a magnetic immunoassaying system has permitted the detection of amyloid beta 42 ($A\beta_{42}$), a promising biomarker of Alzheimer's disease, at the minimum concentration of 23.8 pg/ml. This may enable the identification of the $A\beta_{42}$ levels for the early-stage of Alzheimer's disease with the assistance of the MPI using p-FMMD technique. The results show that the deployment of the p-FMMD can be an alternative to conventional biosensing analytical methods, and can be used as a fast and portable screening method.

1. Introduction

Microfluidics is frequently applied to biosensing when manipulation and analysis of small amounts of biological fluids is conducted in channels with dimensions of less than hundreds of micrometers. The biochip technology has been extensively utilized in various research areas from molecules and cells to organs and medical diagnosis (Dittrich and Manz, 2006; Mazutis et al., 2013; Weigl, 2006). The miniaturized systems have contributed even to the field of chemical reactions with considerable analytic progress (Brivio et al., 2006; Manz, 1990). In many microfluidic systems, the analysis process in biology and medicine is based on fluorescence for sensitive detection by tagging the analytes with fluorescent dye molecules (Ryu et al., 2011). Long-duration experiments require temporally consistent photostability of fluorescent dyes, which is of major importance for generating accurate analysis of targeted biomaterials during experiments (Medintz et al., 2005). However, time-dependent exponential decay of fluorescent dye occurs in many cases, which is caused by irreversible extinction of fluorescent molecules by high energy excitation (Kuwana and Sevick-Muraca, 2002). Quantum dots (Qdots) have recently received attention in a wide range of biological applications to overcome the drawback of conventional fluorescent dyes (Hu et al., 2014). Although Qdots exhibit better photostability and brightness, they also suffer from a blue-shift of their emission under a few minutes of continuous excitation and eventually achieve a photobleached state (Shi et al., 2013). Therefore, tracers that are not affected by excitation sources are preferable for precise analysis because they generate temporally and functionally stable signals and can be regarded as reliable markers.

Magnetic nanoparticles (MNPs) have been widely used in various fields. Among many different types of MNPs, superparamagnetic iron oxide nanoparticles (SPIONs) are especially useful for biological applications because of high coercivity, high magnetic saturation and susceptibility with time-independent characteristics (Reeves and Weaver, 2014). Thus, SPIONs are very practical for a broad range of applications including the detection of specific biological substances, magnetic bio-separation, therapy and targeted drug delivery. Currently, SPIONs also have been

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utilized in medical imaging as image tracers in the relatively new imaging technology called magnetic particle imaging (MPI) (Bauer et al., 2015; Goodwill et al., 2012; Haegele et al., 2012; Hong et al., 2014). MPI is a novel medical imaging method mapping the *in vivo* spatial distribution or local concentration of the MNPs under the appropriate external magnetic fields. Especially, as the SPIONs exhibit nonlinear magnetization characteristics, the MPI technology holds great promise for highly sensitive, and spatially and temporally high-resolution medical imaging (Borgert et al., 2013; Graefe et al., 2015; Panagiotopoulos et al., 2015).

Recently, a new magnetic detection method was developed based on the nonlinear magnetization of superparamagnetic nanoparticles under the magnetic fields generated by two distinct frequencies, called frequency mixing magnetic detection (FMMD) (Krause et al., 2007). When super-paramagnets are exposed to magnetic fields at two distinct frequencies, sum-frequencies representing a linear combination are generated and signalized by lock-in amplification (Krause et al., 2007). The amplified signals are specifically commensurate with the nonlinearity of the magnetization curve of the nanoparticles (Meyer et al., 2007). Based on the FMMD technique, we present a special type of MPI detector for planar samples (p-FMMD). Compared to the existing MPI instrumentation, a sample magnetization is not required for our p-FMMD system because the generated sum-frequency at $f1+2f2$ is maximum at zero static bias field. Therefore, the measurement setup does not need to be bulky in size because of strong magnets. In fact, the dimensions of the measurement head are only 77 mm × 68 mm × 29 mm. The restriction of our system, however, is that only planar samples with a maximum thickness of 2 mm are accessible due to the narrow dimension of measurement slot. The planar sample is scanned by linear translocation within the intermediate space between two measurement heads. A re-construction allowing for thicker samples is possible, but has to be traded in for a loss of spatial resolution. This novel type of scanner allows to obtain images of the distribution of magnetic particles in planar microfluidic biosensing platforms. The results showed the applicability of the p-FMMD technique to a broad range of potential applications such as early diagnosis tool for Alzheimer's disease. In this study, we applied the p-FMMD technique to the quantification of amyloid beta 42, a promising biomarker of Alzheimer's disease, treated on a planar sensing platform as an early diagnosis tool.

2. Materials

2.1. Superparamagnetic nanoparticle and microfluidic platform

In order to study the sensing dimension limits recognizable by the p-FMMD shown in Fig. 1(a), the SPION was purchased from Chemicell (fluidMAG, Germany). The core material is magnetite and the hydrodynamic diameter is about 100 nm. The nanoparticles are dispersed in a storage buffer (ddH_2O, 0.05% sodium azide), having a total weight of volume of 10 μg/μl. In order to consider the effect of the SPION concentration on the measurement resolution of the p-FMMD, 4 different concentrations were prepared for the experiments: 10.0, 5.0, 2.5, and 1.0 μg/μl, diluted with ddH_2O, which correspond to 1.8×10^{10}, 9×10^9, 4.5×10^9, and 1.8×10^9 beads/μl, respectively.

In order to verify the detection limit of a target protein recognizable by the p-FMMD as a magnetic immunoassaying system, amyloid beta 42 ($A\beta_{42}$) was considered as the target protein, which is a promising biomarker of Alzheimer's disease. The $A\beta_{42}$ peptide solutions were diluted to many different concentrations within the ranges from 7.8 pg/ml to 10 ng/ml, as suggested by the manufacturer protocol (Covance, SIG-38956). The primary polyclonal capture antibody was purchased from Invitrogen (44-344, rabbit, USA), and the primary monoclonal biotinylated detection antibody was purchased from Covance (SIG-39144, mouse, USA). The streptavidin-conjugated SPION was purchased from Ocean NanoTech (MHS-50, USA). The core

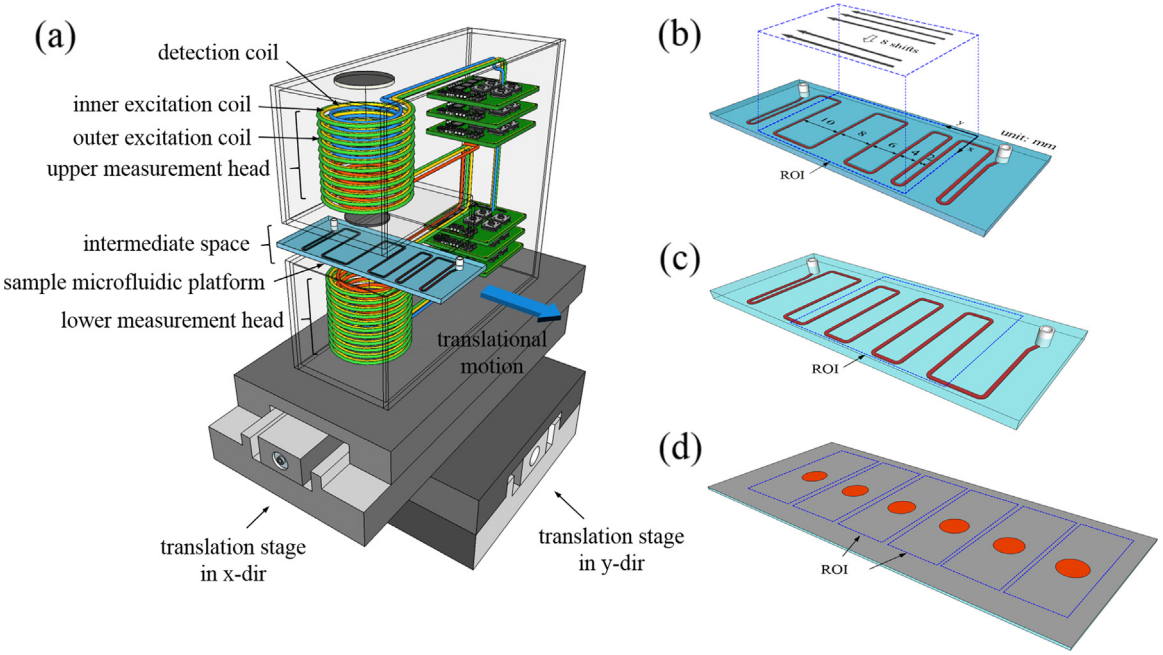

Fig. 1. (a) Schematic diagram of the p-FMMD system composed of the magnetic measurement head and the motorized translation stage. The measurement head is designed in a symmetrical configuration with upper and lower coils, containing a set of two excitation coils and two detection coils in each head. The translation stage is controlled in x and y-direction by an electronic motion controller. (b) A sample microfluidic platform with different widths and intermediate distances. The p-FMMD performs the scanning of the microchannel filled with SPIONs in the range of interest. Arrows indicate the x/y scanning direction during the translational motion of the microfluidic platform. (c) A microchannel with a simple configuration of constant intermediate distance between the adjacent channels. The p-FMMD performs examination of the detection limit for $A\beta_{42}$ peptides. (d) A circular patterned (2 mm in diameter) platform was fabricated for detection of the low concentrations of $A\beta_{42}$ peptides which could not be detected by the microfluidic platforms. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)