

# MICROFLUIDIC APPROACHES FOR MULTIPLEXED IMMUNOCHEMICAL ASSAYS

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## ABSTRACT

This paper reviews our microfluidic approaches for multiplexed immunochemical assays. In particular, topics will be focused on the multiplexed sensing and detection based on the behaviors of immunocomplexed microbeads under magnetophoresis or optoelectrofluidics environment as a new platform for protein sample manipulation and detection. In addition, a microfluidic immunohistochemistry platform will be addressed to give a new insight of biofunctional microfluidics in pathological analysis of breast tissue from patient samples.

**KEYWORDS:** Immunochemical assay, Magnetophoresis, Microfluidic immunohistochemistry, Multiplexed assay, Optoelectrofluidics

## INTRODUCTION

Until now, many approaches for immunochemical analysis have been introduced using an antibody-based molecular detection technology, which ensures the selective, reversible and non-covalent binding of antigens by antibodies. Considering multiple analytes of interest in a fluid sample, assay platforms need their specific multiple detection strategies based on labeling technologies such as fluorescence and nanoparticles. Microfluidics plays a vital role in realizing micro total analysis systems ( $\mu$ TAS) for protein quantification using multiple antibody-antigen reactions. Sample preparation steps can be easily integrated into a microfluidic device to decrease non-specific interactions during immunological reactions of analytes. Owing to a decrease in diffusion limitations at a microscale, immunoreaction time can also be reduced and detection sensitivity may be enhanced by some microfluidic amplification steps. However, there are still several problems such as encoding/decoding problem of multiple analytes, limited availability of specific antibodies, and optical signal detection. Our research focused on the development of microfluidic multiplexed assay technologies based on the microfluidic particle manipulation and detection. Particularly, two manipulation technologies such as magnetophoresis [1–6] and optoelectrofluidics [7–9] have been exploited for the multiplexed detection of protein contents in bloods and cell extracts. In addition, we demonstrated a new analytical concept for multiplexed microfluidic immunohistochemistry [10–12]. These microfluidic platform technologies will facilitate the development of diagnostic tools for personalized medicine.

## MAGNETOPHORETIC IMMUNOASSAYS

Magnetophoresis is a phenomenon explaining particle migration driven by magnetic force exerted on a particle. In a microchannel, magnetic force induced by an external magnetic field exerts an object to move toward the denser or sparser magnetic field according to the magnetic susceptibility difference between particle and its surrounding fluid (Figure 1a). Previously, our group has developed a new immunoassay method based on the magnetophoretic mobility of a microbead, depending on the amount of associated superparamagnetic nanoparticles under magnetic field gradient in a microfluidic channel [1–4]. By measuring the magnetophoretic deflection velocity or positions of microbeads in a microchannel as the signal for the presence of analytes, the multiple disease biomarkers were simultaneously quantified by conjugated magnetic nanoparticles. An improved magnetophoresis technique, called isomagnetophoresis, was also developed to discriminate subtle differences in magnetic susceptibility by using a magnetic susceptibility gradient in a microfluidic channel [5]. In isomagnetophoretic immunoassays, the magnetic nanoparticles were used as labels on microbeads in sandwich-type immunoassay, detecting the amount of bound analytes by isomagnetophoretic focusing the immunocomplexed microbeads under the magnetic

susceptibility gradient and magnetic field in a microchannel. This immunoassay can be useful to accurately quantify the concentrations of breast cancer biomarkers over the whole range of analyte concentrations in patient samples [6].

### OPTOELECTROFLUIDIC IMMUNOASSAYS

Optoelectrofluidic technology allows programmable manipulation of particles or fluids in microenvironments based on optically induced electrokinetics resulted from photochemical, photoconductive, and photothermal effects (Figure 1b). Previously, we demonstrated a rapid and selective concentration of micro- and nanoparticles or cells by combining several electrokinetic mechanisms, including ac electroosmosis and dielectrophoresis, and electrostatic interactions [7,8]. A new kind of optoelectrofluidic sandwich immunoassay scheme was also proposed using an optoelectrofluidic device coupled with conventional surface-enhanced Raman scattering (SERS) setup [9]. This image-driven immunoassay platform opens a new way for simple, fast, automated, and highly sensitive detection of antigens without any washing steps. Currently, we are committed to making further progress in developing a multiplexed optoelectrofluidic immunoassay platform combined with conventional microarray format.

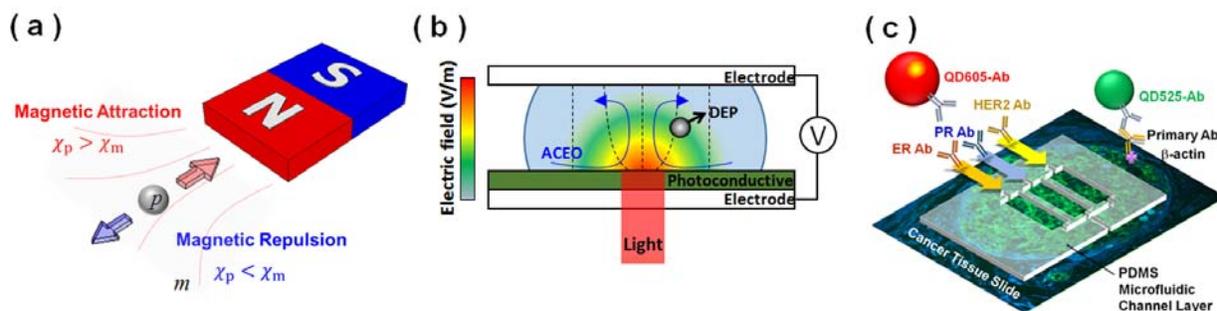


Figure 1: Schematics of microfluidic manipulation principles. (a) Magnetophoresis is defined as a phenomenon which describes the particle migration that occurs when a magnetic force is exerted on a particle. Here, the movement of particle ( $p$ ) depends on the relative strength of magnetic susceptibility of a particle ( $\chi_p$ ) over magnetic susceptibility of a fluid ( $\chi_m$ ). (b) Optoelectrofluidics describes the motion of particles or molecules and their interactions with an optically induced electric field and surrounding fluid. The particle movements in a liquid chamber are driven by several frequency-dependent optoelectrofluidic phenomena such as optically induced ac electroosmosis (ACEO) and dielectrophoresis (DEP). (c) Quantum dot (QD)-based microfluidic multiple biomarker quantification method. By reversible bonding of a PDMS upper layer with multiple channels on the tissue slide directly, microfluidic immunostaining allows simultaneous incubation with multiple antibodies (Abs), producing well-defined strip-type staining results of breast cancer biomarkers, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).

### MICROFLUIDIC IMMUNOHISTOCHEMISTRY

Immunochemical assay can provide information on both cellular morphology and the quantities of molecules within cells or tissues. Particularly, in immunohistochemistry field, multiplexed protein quantification remains difficult using conventional methods. We developed a PDMS-based microfluidic interface that enables multiplexed immunohistochemistry (IHC) measurements on breast tissue samples [10,11]. By directly pressing the PDMS device onto a conventional thin-sectioned tissue slide, we can form simple and robust microchannels for perfect fluid control for immunostaining solutions, without any leakage, bubble formation, or cross-contamination. This new IHC platform has improved performance on assay time, consumption of tissue, antibodies and staining compounds, sensitivity, specificity, and cost-effectiveness. Recently, we also developed a microfluidic double-staining method based on quantum dots

(Figure 1c), resulting in accurate quantification by normalization of biomarker levels to that of internal reference protein [12]. This result indicates that the microfluidic IHC platform is useful for accurate histopathological diagnosis using numerous specific biomarkers simultaneously.

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