DNA detection methods based on microbeads dielectrophoresis

丁, 震昊

（別紙様式 2）

氏　　名　：丁　震　昊

論 文 名　：DNA detection methods based on microbeads dielectrophoresis
（微粒子誘電泳動を利用した DNA 検出法）

区　　分　：甲

論　文　内　容　の　要　旨

The early detection and identification of a bacterial caused food contamination or infectious disease is required to select an appropriate clinical treatment or to assess the danger to the public. Genetic methods such as the polymerase chain reaction (PCR) have been used for bacterial detection. The high sensitivity, specificity, and speed of PCR-based methods have led to the development of DNA-based bacterial detection methods. DNA amplified by PCR, are generally separated by size and detected by agarose gel electrophoresis. Although this method is well established and reliable, it requires rather complicated and time-consuming manual operations, carried out by experts. Hence, it is important to develop a more rapid, simple, cost-effective, and sensitive method for the detection of amplified DNA.

This research focuses on the dielectrophoresis (DEP) characteristic alteration when DNA was attached to the microbeads. When DNA molecules are chemically attached to the surface of dielectric microbeads, the DNA functionalization alters the surface conductance of microbeads and result in the change of Clausius–Mossotti (CM) factor K of DEP, which affects the DEP force. Since the surface conductance of DNA labeled microbeads will increase along with the attached amount of DNA, the DEP force act on the DNA labeled microbeads, which is related to the surface conductance of DNA labeled microbeads, changes along with the attached amount of DNA. Therefore, we can detect the DNA by monitoring the changes of DEP force act on the DNA labeled microbeads. We developed three DNA detection methods based on the positive DEP (p-DEP), negative DEP (n-DEP) and traveling wave DEP (twDEP), respectively.

Method 1: Microbeads positive dielectrophoresis-based DNA detection.

In this method, we focus on the p-DEP of DNA labeled microbeads. When large enough amount of DNA attached to one microbead, the DEP force act on the DNA labeled microbead change from negative to positive. Under p-DEP force, the DNA-labeled microbeads will be trapped on a microelectrode, whereas the pristine ones, which will experience n-DEP, will be repelled from the electrode. Combining this dramatic alteration in DEP phenomena with impedance measurement allows rapid and quantitative detection of the amplicons. In order to develop this DNA detection method, we studied how DNA labeling affect the microbeads DEP characteristic by investigate the crossover frequency of DNA labeled microbeads and also studied the DNA detection ability of this novel electrical detection method by using a template DNA. Furthermore, we applied this method for bacterial detection by detecting different concentration of *E. coli* solution and also by selectively detecting *E. coli* from mix solution of *E. coli* and yeast.

Method 2: Microbeads negative dielectrophoresis-based DNA detection.

In order to change the DEP behavior of microbeads from negative to positive, large amount of DNA have to be labeled onto one microbead. Although less amount of DNA labeling will not alter the DEP behavior of microbead from negative to positive, the particle surface conductance

will still change due to the negative charge of labeled DNA, hence the change of the n-DEP force. Therefore, more sensitive detection of DNA can be achieved by distinguish the microbeads experiencing different n-DEP force caused by DNA labeling. Hence, we propose a microfluidic device with parallel ITO electrode pair and combined it with fluorescent detection for sensitive DNA detection. This method is based on the change of the microbeads trajectories in the fluidic device due to the change of experienced n-DEP force. The trajectories of microbeads were firstly simulated. Then DNA labeled fluorescent microbeads were flow into the proposed fluidic device and the trajectories of the DNA labeled fluorescent were observed. Then, the fluorescent of microbeads was amplified by a photomultiplier and measured by a data logger to distinguish DNA concentration.

Method 3: Microbeads traveling wave dielectrophoresis-based DNA detection.

Although the detection method based on the n-DEP allows sensitive detection of DNA, it requires the use of fluorescent microbeads. Furthermore, this method requires the use of the microfluidic device, which requires rather complex operation. Therefore, we proposed a new sensitive DNA detection method based on the twDEP, which is a phenomenon affected by the imaginary part of K (Im[$\tilde{K}$]). Theoretically, the Im[$\tilde{K}$] changed more dramatically than Re[$\tilde{K}$] when microbeads surface conductance is small, which means Im[$\tilde{K}$] will change more dramatically when less DNA was labeled on the microbeads. Since the velocity of microbeads will change due to different twDEP forces, we measured the velocity of DNA labeled microbeads under twDEP force using computer-based image analysis.

The whole thesis is composed of seven chapters:

Chapter 1 gives background as well as some basic introduction of bacterial detection.

In chapter two, the principles of DEP and DEPIM method are described.

In chapter three, the DNA detection method based on p-DEP by using template DNA was demonstrated.

In chapter four, the p-DEP based DNA detection method is applied to bacterial detection by detecting different concentration of *E. coli* solution and also by selectively detecting *E. coli* from mix solution of *E. coli* and yeast.

In chapter five, we demonstrate a sensitive DNA detection method based on n-DEP. The trajectories of DNA labeled microbeads inside the designed microfluidics are studied and measured by fluorescent measurement.

In chapter six, a simple and sensitive DNA detection method based on twDEP is investigated. The velocity of DNA labeled microbeads, which is affected by the twDEP force was studied.

Chapter 7 summarizes this thesis by comparing these three methods.