

Abstract

Ultra-Sensitive Immunofluorescence Assay Based on DNA Elongation by Surface Plasmon Heating [†]

Yuki Kawahara and Akito Ishida *

Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan; s816631012@kpu.ac.jp

* Correspondence: a_ishida@kpu.ac.jp

[†] Presented at the 5th International Symposium on Sensor Science (I3S 2017), Barcelona, Spain, 27–29 September 2017.

Published: 11 December 2017

We present here a novel ultra-sensitive immunological fluorescent method of assaying bacterial and viral pathogens. The assay is based on the selective heating of a target binding-site by the surface plasmon (SP) that is induced on a flat gold film in response to excitation with a high-power, near-infrared laser light. This ‘localized’ heating induces rolling-circle DNA amplification (RCA; i.e., continuous isothermal DNA elongation using a circular template) at the pathogen binding-site. The resulting DNA amplicon can then be detected via fluorescent staining.

To enable selective heating of the target binding-site, a primer sequence-modified antibody is first immobilized on the surface of the gold film as a spot with which to capture target pathogens. Since a pathogen-bound antibody produces a larger SPR angle (q') than a free antibody (q), SP fields are generated at the binding-site in response to laser illumination at a slightly offset incident angle ($q'' = q' + q_{\text{offset}}$) from the q' . The SP damping heat can then induce selective DNA elongation from the primers immobilized on the antibodies binding pathogens. This allows pathogens to be covered by the amplicons, and produces large DNA spheres that can be fluorescently stained, and then easily visualized and counted using a low-magnification microscope or imager.

In the present study, we used 3- μm IgG-conjugated latex beads as a model of pathogen and confirm the validity of the assay. Anti-IgG and anti-BSA (control) antibodies were modified by 5'-NH₂-M13M4-primers using bis-NHS linkers. A 6-mm silicon rubber through-hole well was then attached to a 50-nm gold film that had been evaporated onto a coverslip. The two antibody conjugates were immobilized on the NHS-terminated gold surface to form 1-mm test and control spots, respectively. After immuno-reaction with the model particles, the surface was immersed in an RCA solution (comprising M13mp18, *Bst* polymerase, and dNTPs), and illuminated by a p-polarized 808-nm 600-mW laser light for 30 min through a glass prism, and at the incident angle (q''). The resulting amplicons were stained with SYBR Green-I, and observed via confocal microscopy ($\times 40$ magnification). A strong fluorescent signal was observed specifically at the test spot. The locations of the fluorescence spots were consistent with those of the particles in the transmission image at higher magnification. This result indicates that SP heating successfully induced RCA at the binding site of the 3- μm latex beads.

Although the observed background fluorescence intensity at the control spot was significantly lower than that at the test spot in the present study, non-specific heating and/or background RCA could decrease image contrast in other instances. Thus, setting the offset angle (θ_{offset}) will likely be crucial during practical application of this method, particularly if the assay is used to detect viral pathogens that only generate a small SPR angle change ($d\theta = \theta' - \theta$) upon binding to the antibody. The optimization of the offset angles for various size pathogens will therefore be discussed.



© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).