スマートフォン蛍光顕微鏡を用いた単一ウィルスのデジタルバイオアッセイ

A single virus digital bioassay using smartphone fluorescence microscopy

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Point of care testing (POCT) is a test that conducted near side of patient whenever medical care is needed. Therefore, rapid and simple diagnosis is desirable features for POCT. Digitalization of fluorogenic enzymatic assays through the use of femtoliter-volume reactor arrays is an emerging approach to realizing highly quantitative and rapid bioassays. However, to observe the fluorescence signal in femtoliter-volume compartment, we require expensive and large microscope system. To apply the digital bioassay to POCT, it is necessary to develop portable and simple observation system. Here we demonstrate a single virus digital bioassay using smart phone fluorescence microscopy.

Firstly, we achieved a single influenza virus digital assay. When single influenza virus were encapsulated into the femtoliter-volume reactor arrays at a low ratio of less than 1 molecule per reactor, each reactor showed a discrete fluorescence signal in an all-or-none manner, allowing the digital counting of the number of active influenza viruses.

Then, we developed a compact fluorescence observation system employing smartphone. This system involves a compact laser diode to illuminate femtoliter-volume reactor arrays with evanescent field, a long-pass thin film filter, aspherical lens, and the mechanical translation stage for observing a large number of reactors (Fig.1). When the light originating from the laser diode undergoes total internal refection within the glass on the femtoliter-volume reactor, the evanescent field is generated. We tested the imaging performance of this smartphone fluorescence microscopy system by conducting the single virus digital bioassay. We detected a discrete fluorescence signal in an all-or-none manner. For comparison, the same sample was imaged with conventional fluorescence microscopy. The position of bright reactor within same area was corresponds to each other (Fig.2). This result suggests that the fluorescence microscopy employing smartphone permit the digital bioassay to POCT application.

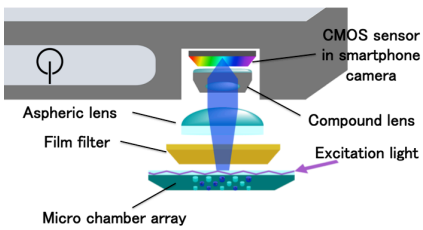

Fig.1 Schematic image of smartphone fluorescence microscopy

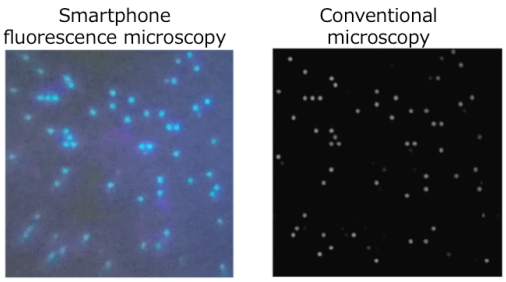

Fig.2 Comparison of smartphone fluorescence microscopy and conventional microscopy