

APPLICATION OF FLIM/FRET FOR THE DETECTION OF EV71 VIRUS INFECTION IN CELLS

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Timely and effective virus infection detection is critical for the clinical management and prevention of the disease spread in communities during an outbreak. A range of methods have been developed for this purpose, of which classical serological and viral nucleic acids detection are the most popular. We describe an alternative imaging based approach for the detection of Enterovirus 71 (EV71) infection that utilizes fluorescence resonance energy transfer (FRET) resolved by fluorescence lifetime imaging microscopy (FLIM). A plasmid construct was developed with the sequence for GFP2 and dsRed2 fluorescent proteins, linked by a 12- amino acid long cleavage recognition site for the 2A^{pro} protease, encoded by the EV71 genome and specific for the members of *Picornaviridae* family. When expressed in HeLa cells the spacer bound the fluorophores within the Förster distance and created a condition for FRET to occur that resulted in the shortening of GFP2 fluorescence lifetime. Upon cells infection with EV71, the 2A^{pro} released to the cytoplasm clove the recognition site and caused the disruption of FRET through separation of the fluorophores. The increased GFP2 lifetime, manifested by the time correlated single photon counting, presents a timely and effective way in detecting virus infection.

