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

Fu-Jen Kao¹,³, Yueh-Ying Hsu², Szu-Hao Kung², Vladimir Gukassyan¹,
¹Institute of Biophotonics, National Yang-Ming University, Taipei 11221, Taiwan
²Institute of Biotechnology in Medicine, National Yang-Ming University, Taipei 11221, Taiwan
³Taipei City Hospital, Taipei 10341, Taiwan
E-mail: fujenkao@ms21.hinet.net

Keywords: FLIM, FRET, virus infection, GFP2

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 2Apro 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 2Apro 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.

Figure 1. Color coded lifetime images (lifetime measured in the GFP channel (526nm ±23nm)): a. G2AwtR in mock HeLa cells, b – G2AwtR in EV71 infected HeLa cells, c. G2wtR in cisplatin treated HeLa cells, d. G2AwtR in EV71 infected HeLa cells, e. G2AwtR in HSV infected HeLa cells, f. the corresponding lifetime histograms.