Real-time confocal fluorescence lifetime imaging microscopy (FLIM) based on the analog mean-delay (AMD) method

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Fluorescence lifetime imaging microscopy (FLIM) is a powerful functional imaging technique that has been applied in many bio-medical research fields to visualize localized environmental conditions, such as pH, ion concentration, refractive index, and the occurrence of fluorescence resonance energy transfer (FRET) [1–2]. We have demonstrated GPU accelerated real-time confocal FLIM based on the AMD method. Clear imaging was achieved by perfect synchronization between the x-y scanner, pulse laser, and digitizer by using a DAQ board and a D-FF digital circuit sync block. Data from the digitizer was copied to the rolling buffer of the host memory such that real-time data processing was possible without any data loss. For the confocal AMD-FLIM with 4 kHz resonant scanner, GPU processing time was faster than physical scanning time up to image sizes of 800 × 800 pixels, and was more than 149 times faster than single core CPU processing times. We demonstrated the total frame rate of our system by observing maize vascular tissue. We achieved a frame rate of about 13 fps for an image of 200 × 200 pixels. This system can be utilized for observing dynamic biological reactions, medical diagnosis, and real-time industrial inspection.

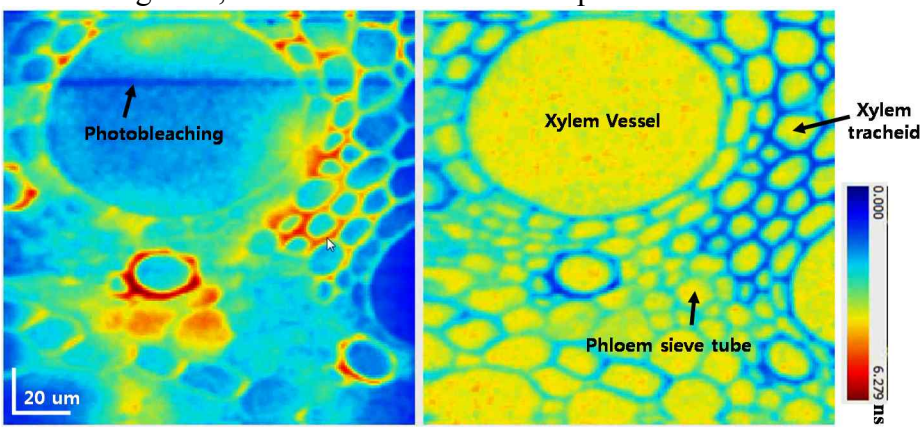

Fig. Real-time fluorescence lifetime imaging of maize vascular tissue based on the analog mean-delay (AMD) method (a) fluorescence intensity image (b) fluorescence lifetime image

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[2] J. Neefjes, and N. P. Dantuma, "Fluorescent probes for proteolysis: tools for drug discovery," Nat. Rev. Drug Discov. **3**(1), 58–69 (2004).

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