Confocal Imaging Fluorometer: Detection of Chlorophyll Fluorescence Transient *in vivo* with High Spatiotemporal Resolution

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Chlorophyll fluorescence (CF) is a powerful tool for plant physiology study since it has been proven to be strongly associated with photosynthetic reaction [1]. Conventional fluorometers such as plant efficiency analyzer (PEA) and pulse amplitude modulation (PAM), provide a convenient approach to characterize CF. However, they only measure ensemble effects through wide-field imaging modality, with millimeter spatial and sub-second temporal resolutions. In this work, we demonstrated a confocal imaging fluorometer, which offers much higher spatiotemporal resolutions, allowing successful determination of CF transient (i.e. the Kautsky curve) from individual chloroplast. As shown in Fig. 1, both the temporal dynamics and the intensity dependences correspond well to the ensemble measurement from conventional studies, but the individual variation between chloroplasts is only differentiable with confocal techniques. Different decay life time (i.e., the half-life period of the slow decay in the Kautsky curve) represents different level of photosynthetic conditions. This result opens up new possibilities toward detailed spatial analysis of photosynthesis on the scale of organelles.

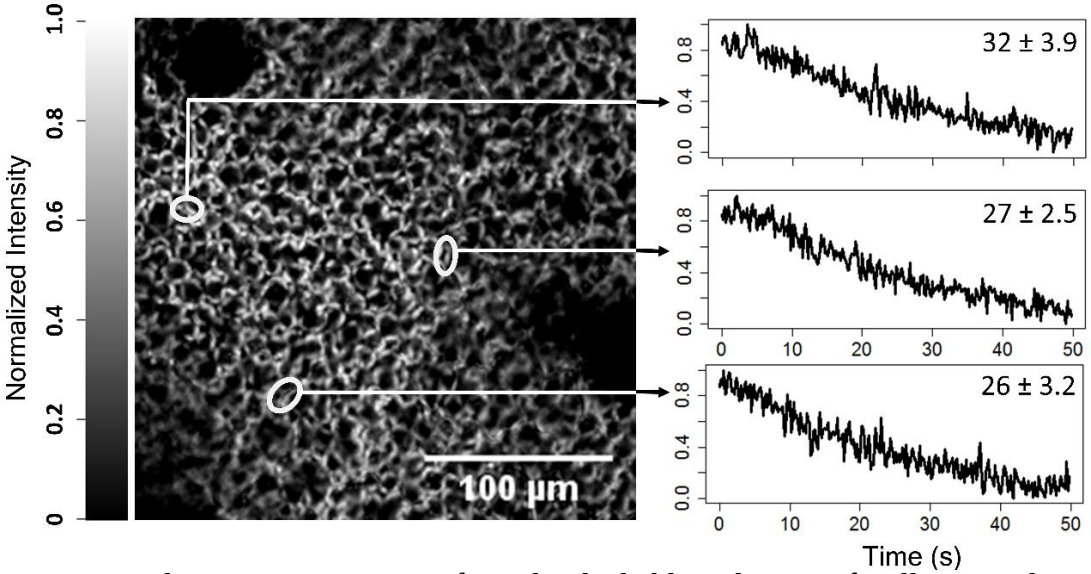

Figure 1: Fluorescence transients for individual chloroplasts confocally imaged at 40 kW/cm^2.

[1] Murchie, E.H. and T. Lawson. **(2013)**. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *Journal of Experimental Botany*. 64(13):3983-3998.