

Supplementary Information to Nondestructive circadian profiling of starch content in fresh intact *Arabidopsis* leaf with two-photon fluorescence and second-harmonic generation imaging.

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Empirical determination of threshold value for pixels containing chloroplast

Fig. S1 compares the TPF images threshold at different values. The first column shows TPF images of Col-0 leaves at ZT0, ZT6, ZT15, and ZT21 with intensity scale set to 0 - 200. The columns to the right are the same image displayed as a binary image with the threshold value shown on top. A threshold value set too high leads to unaccounted pixels containing chloroplast, while a threshold value set too low would over count the area covered by chloroplast. Based on the images, we chose a threshold value of 50 to determine the number of pixels containing chloroplast. The sum of the pixels above the threshold value is the Cnt_{TPF} value used to calculate $I_{\text{SHG}}/\text{Cnt}_{\text{TPF}}$ in Fig. 5 of the main text.

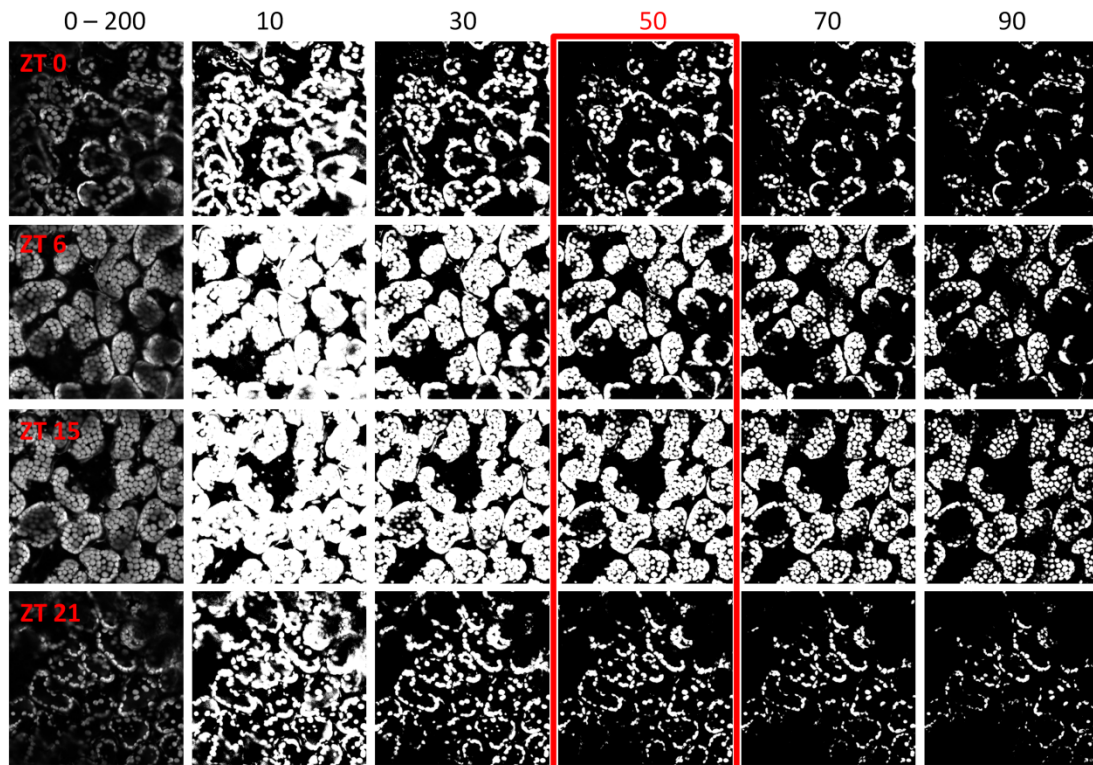


Figure S1 Empirical determination of TPF count threshold for statistical analysis.