Supplementary Materials

Fit-derived phasor analysis of metabolic signatures of cerebral endothelial dysfunction in Alzheimer's disease: a proof-of-concept study

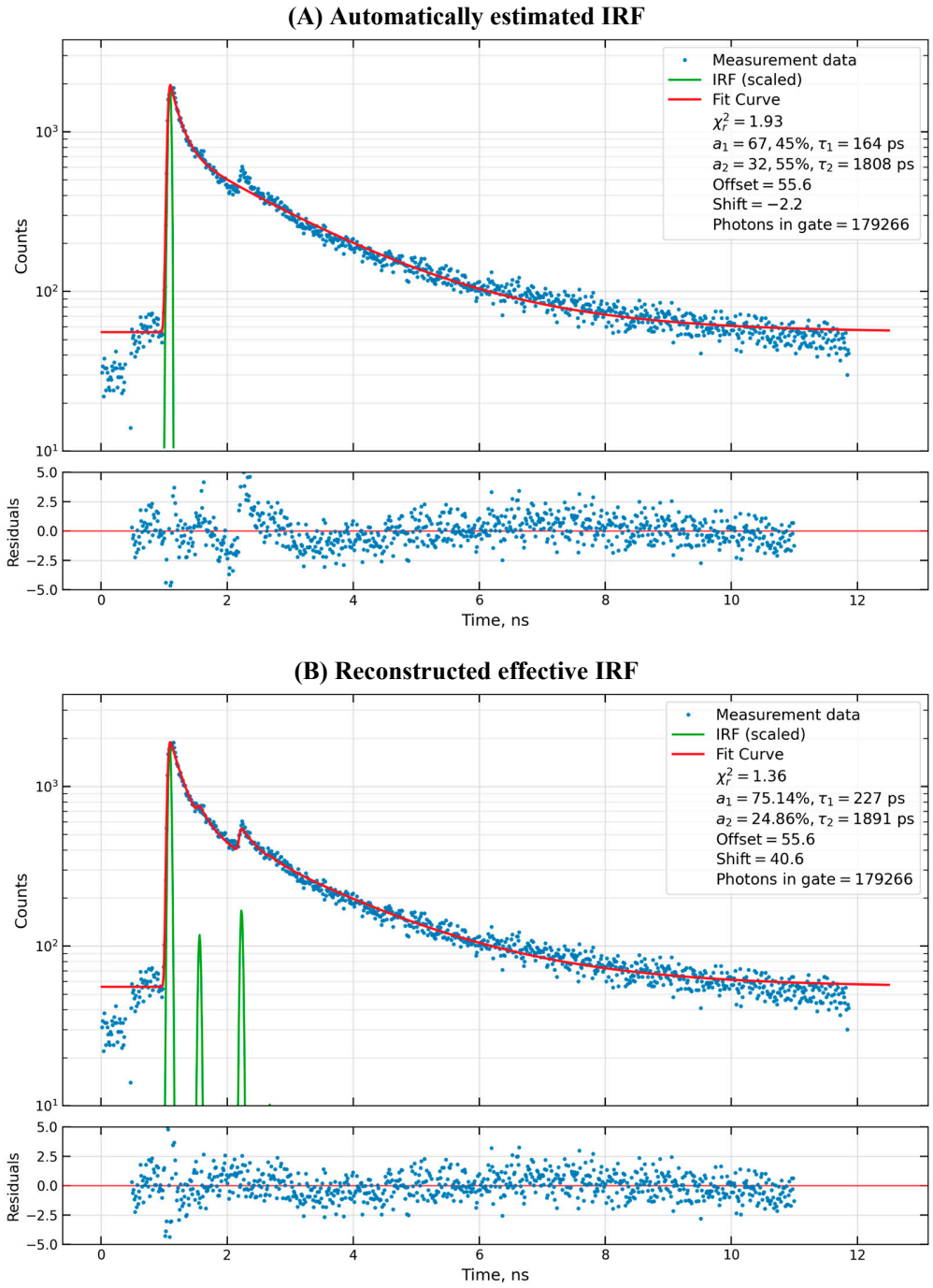


Figure S1. Effect of IRF treatment on biexponential fitting. (A) IRF estimated automatically in SPCImage. (B) Reconstructed effective IRF accounting for additional delayed peaks. Both panels show the same cellular decay, containing 179,266 photons within the selected time gate. Blue points denote the experimental data, red lines the reconvolved fits, and green lines the IRFs, scaled for display. Fitting residuals are shown below each decay. The IRFs were reconstructed from fluorescence data rather than measured independently; the main peak of the effective IRF had an FWHM of 50 ± 10 ps. The reduced χ^2 decreased from 1.93 to 1.36 with the reconstructed IRF.

Monoexponential and biexponential decay fitting

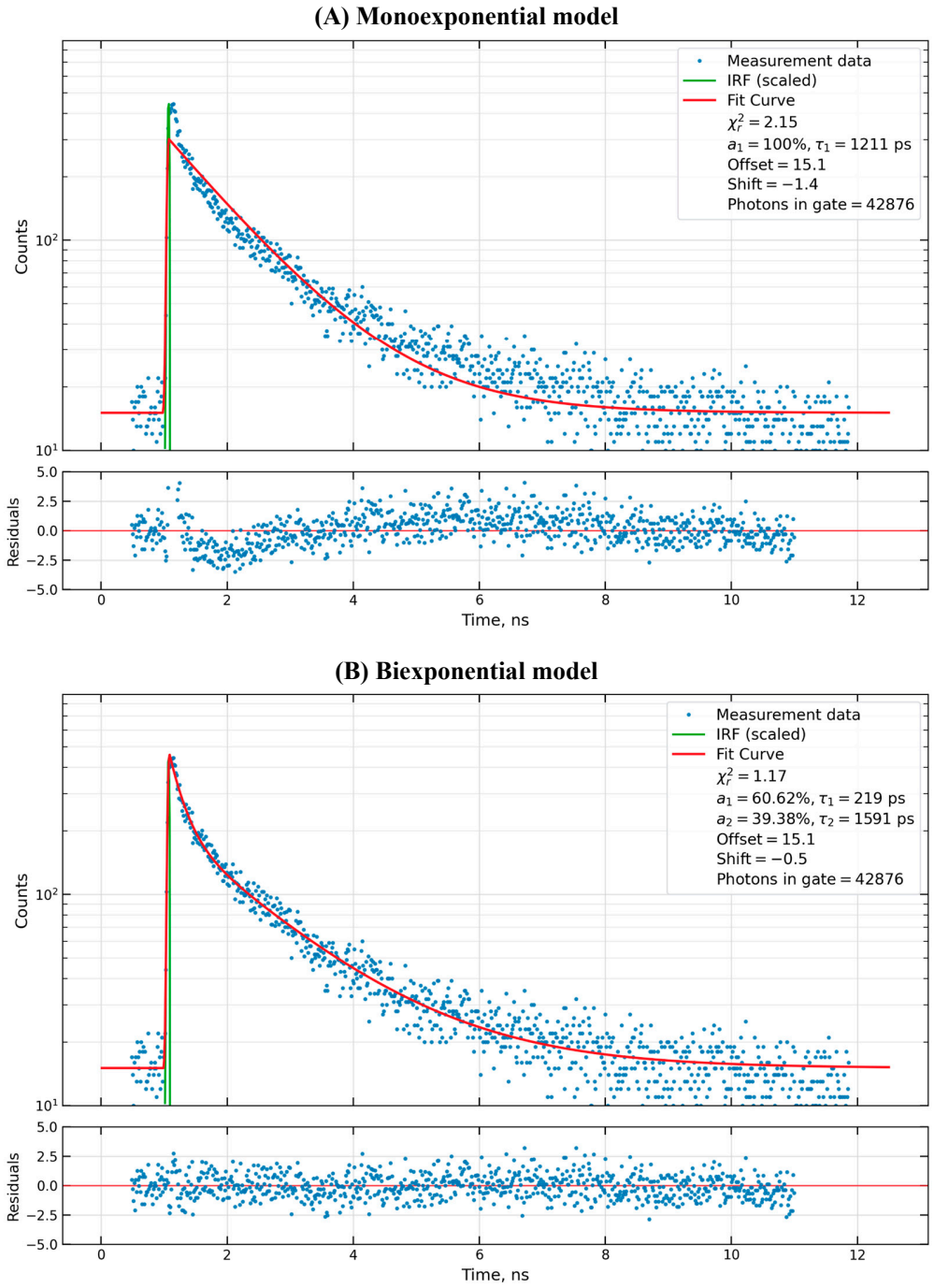


Figure S2. Comparison of monoexponential and biexponential fits to the same cellular decay, containing 42,876 photons within the selected time gate. (A) Monoexponential fit. (B) Biexponential fit. The IRF was estimated automatically in SPCImage for both models. Blue points denote the experimental data, red lines the reconvolved fits, and green lines the IRFs, scaled for display. Fitting residuals are shown below each decay. The reduced χ^2 decreased from 2.15 to 1.17 with the biexponential model. The fitted lifetimes were 1211 ps for the monoexponential model and $\tau_1 = 219$ ps and $\tau_2 = 1591$ ps for the biexponential model, with relative amplitudes of 60.62% and 39.38%, respectively.

Long lifetime component of NAD(P)H fluorescence

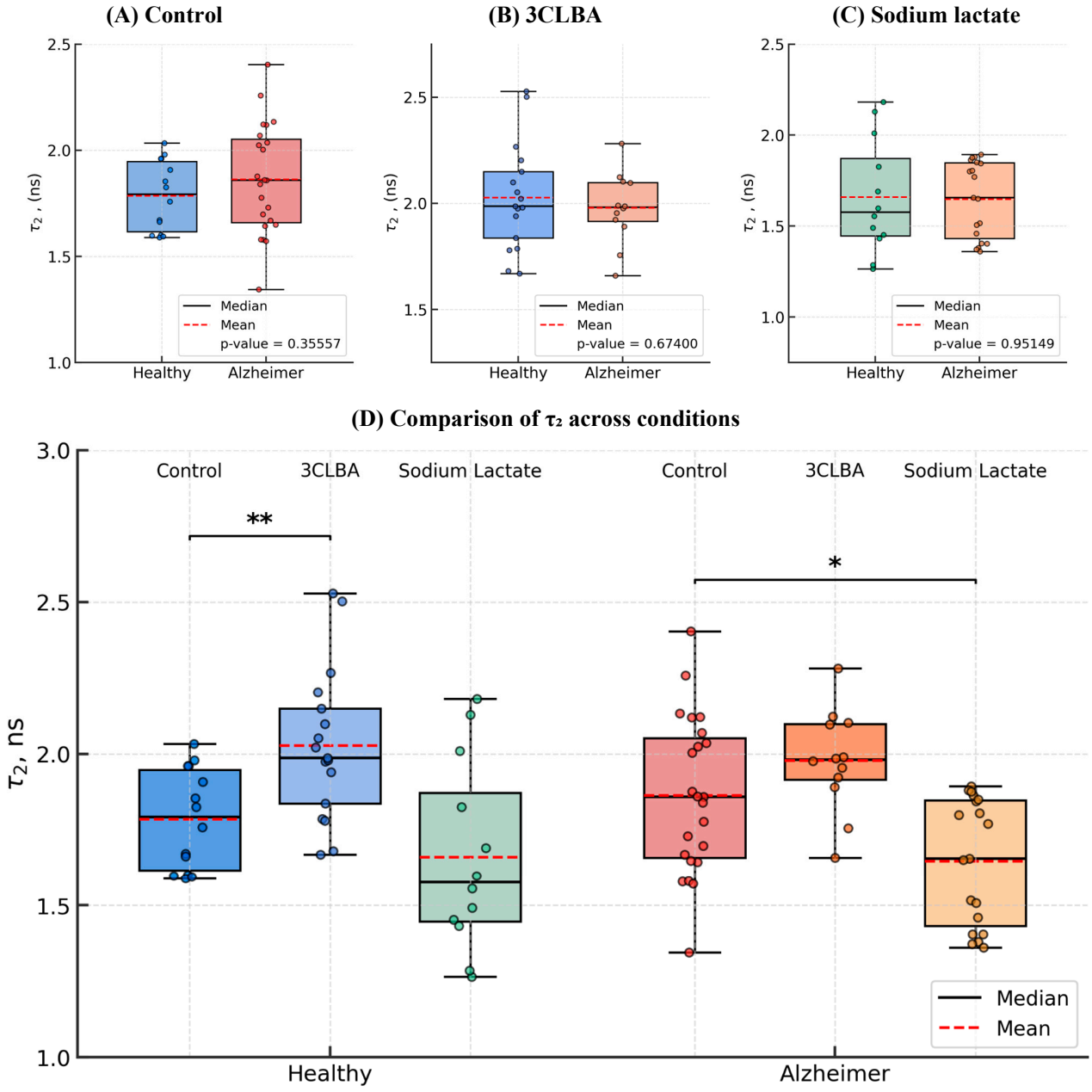


Figure S3. Long-lifetime component τ_2 of NAD(P)H fluorescence in PO2 (Healthy) and Alz9L (Alzheimer) cells. (A–C) Comparisons between the cell lines under control conditions, after 3CLBA treatment, and after sodium lactate treatment, respectively. The corresponding p-values were 0.35557, 0.67400, and 0.95149. (D) Distributions across conditions within each cell line. Each point represents an individual cell; solid black lines indicate medians and dashed red lines indicate means. Pairwise comparisons used the Mann–Whitney U test, as described in the Methods. Asterisks indicate the marked within-line comparisons: *$p < 0.05$; **$p < 0.01$. Cell-level p-values are nominal and exploratory because the tests did not account for the nesting of cells within fields of view and wells.

Comparison of fit-derived and SPCImage phasor representations of NAD(P)H fluorescence decays under basal conditions

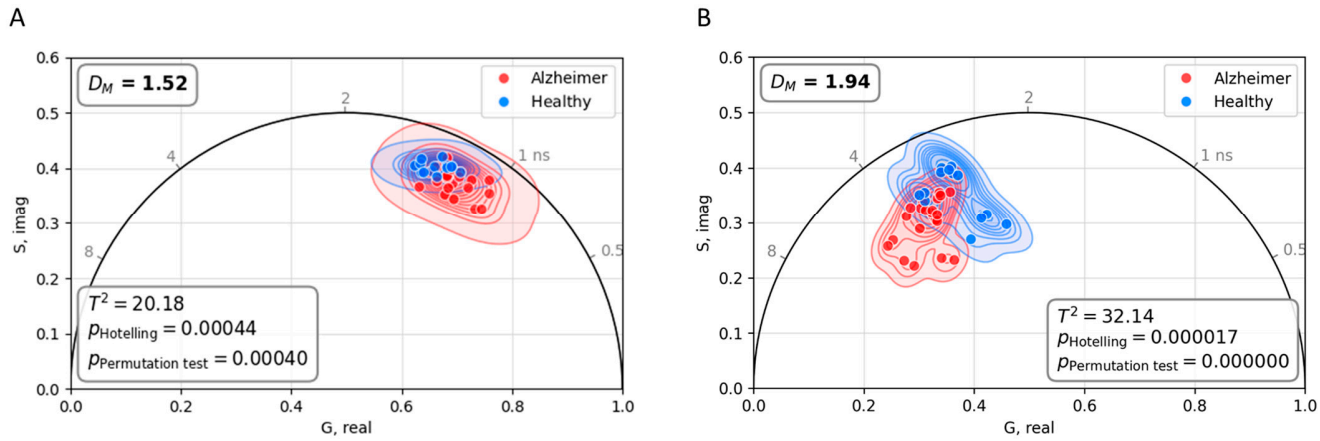


Figure S4. Comparison of fit-derived and SPCImage phasor representations of NAD(P)H fluorescence decays under basal conditions. (A) Phasor coordinates reconstructed from the biexponential fitting parameters, as presented in the main manuscript. (B) Phasor coordinates obtained from the experimental TCSPC decays using the SPCImage phasor routine with the reconstructed effective IRF supplied to the software. Each point represents one cell, and contours indicate kernel density estimates. Both representations were calculated at the laser repetition frequency of 80 MHz. Group separation was observed in both analyses, although the absolute phasor coordinates differed substantially between the two representations.