

## DUAL-EXCITATION MULTISPECTRAL FLUORESCENCE LIFETIME IMAGING HANDHELD ENDOSCOPE FOR LABEL-FREE METABOLIC AND BIOCHEMICAL IMAGING OF THE ORAL MUCOSA: SUPPLEMENTAL DOCUMENT

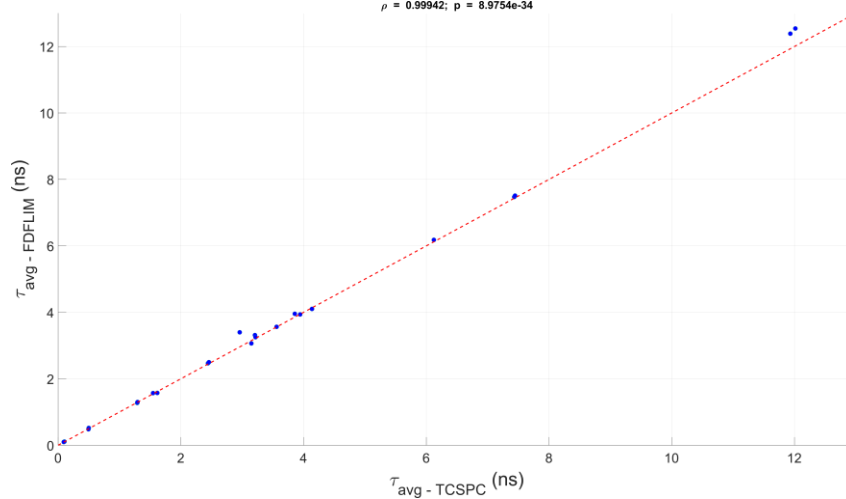
In this supplementary material, we delve into the statistical analysis of the average lifetimes of fluorescence standards measured with the FD-FLIM system, compared to those measured with a calibrated Time-Correlated Single-Photon Counting (TCSPC) spectrometer which were summarized in **Table 1** of the Letter.

The following fluorescence standard solutions were prepared using Ethanol (EtOH), phosphate buffered saline (PBS, Sigma Aldrich P5368), Methanol (MeOH), and deionized water (DI Water) as solvents: POPOP (Sigma Aldrich P3754), NADH (Sigma Aldrich 10107735001), Anthracene (ANT, Sigma Aldrich A3885), DPA (Sigma Aldrich D205001), Coumarin 1 (C1, Sigma Aldrich D87759), 9-Anthracenecarbonitrile (9CA, Sigma Aldrich 152765), Rhodamine B (Sigma Aldrich 83689), Rose Bengal (Sigma Aldrich 330000), fluorescein sodium salt (Flu, Sigma Aldrich F6377), FAD (Sigma Aldrich F6625), Coumarin 6 (C6, Sigma Aldrich 442631), and Rubrene (Sigma Aldrich 554073). Detailed information on the preparation of the fluorescence standard solutions, including their molar concentrations ([M]) and respective solvents, is provided in **Table S1**. Additionally, **Table S1** summarizes the average lifetimes and average normalized multispectral fluorescence intensities, measured at specific excitation wavelengths ( $\lambda_{ex}$ ) and corresponding emission spectral channels ( $\lambda_{em}$ ) for each fluorescence standard solutions.

**Table S1 – Detailed Information on Fluorescence Standard Solutions**

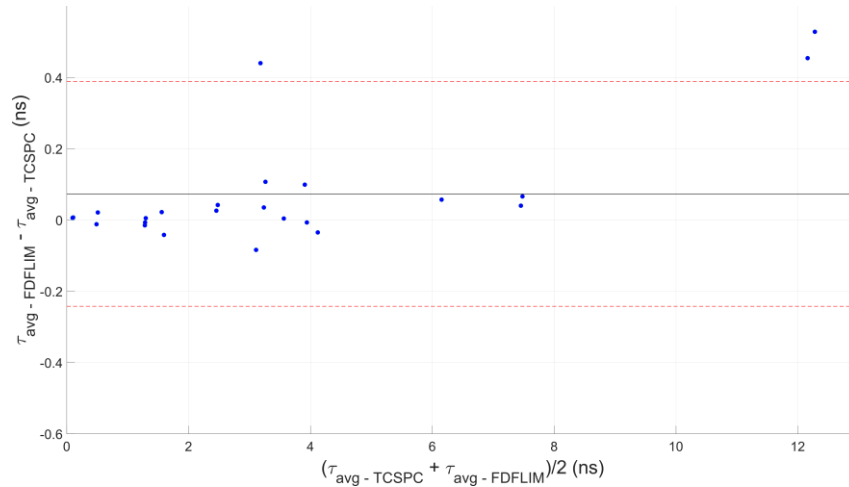
Fluorophore	Solvent	[M] ( $\mu$ M)	$\lambda_{ex}$ (nm)	$\lambda_{em}$ (nm)	Norm. M.S- Intensity	$\tau_{avg-TCSPC}$ (ns)	$\tau_{avg-FDFLIM}$ (ns)
POPOP	EtOH	0.1	375	405 $\pm$ 20	0.46	1.289	1.274 $\pm$ 0.009
				482 $\pm$ 18	0.37	1.289	1.282 $\pm$ 0.014
				550 $\pm$ 44	0.16	1.294	1.299 $\pm$ 0.031
NADH	PBS	80	375	482 $\pm$ 18	0.37	0.499	0.520 $\pm$ 0.011
				550 $\pm$ 44	0.54	0.494	0.482 $\pm$ 0.009
ANT	EtOH	1	375	405 $\pm$ 20	0.84	4.137	4.102 $\pm$ 0.059
DPA	EtOH	1	375	405 $\pm$ 20	0.57	6.123	6.180 $\pm$ 0.063
Coumarin 1	EtOH	1	375	405 $\pm$ 20	0.07	3.149	3.065 $\pm$ 0.068
				482 $\pm$ 18	0.63	3.207	3.314 $\pm$ 0.022
				550 $\pm$ 44	0.29	3.217	3.252 $\pm$ 0.040
9CA	EtOH	5	375	482 $\pm$ 18	0.52	12.014	12.542 $\pm$ 0.121
				550 $\pm$ 44	0.31	11.932	12.386 $\pm$ 0.148
Rhodamine B	DI Water	1.25	375	550 $\pm$ 44	0.72	1.616	1.574 $\pm$ 0.032
			445		0.73	1.547	1.569 $\pm$ 0.063
Rose Bengal	PBS	50	375	647 $\pm$ 28	0.29	0.102	0.109 $\pm$ 0.072
			445		0.28	0.092	0.098 $\pm$ 0.007
Fluorescein	PBS	0.05	445	482 $\pm$ 18	0.10	3.857	3.956 $\pm$ 0.089
				550 $\pm$ 44	0.87	3.944	3.937 $\pm$ 0.047
FAD	PBS	5	445	550 $\pm$ 44	0.83	2.959	3.399 $\pm$ 0.093
				647 $\pm$ 28	0.13	3.560	3.564 $\pm$ 0.048
Coumarin 6	EtOH	0.02	445	482 $\pm$ 18	0.25	2.442	2.468 $\pm$ 0.009
				550 $\pm$ 44	0.73	2.457	2.499 $\pm$ 0.019
Rubrene	MeOH	< 8	445	550 $\pm$ 44	0.64	7.433	7.473 $\pm$ 0.049
				647 $\pm$ 28	0.22	7.446	7.512 $\pm$ 0.094

To ensure the robustness of our data, we employed two complementary statistical methods: correlation analysis and Bland-Altman plots. By applying correlation analysis to examine the agreement between the average lifetimes measured by the two systems, we obtained a linear correlation coefficient of  $\rho = 0.99942$ . This result indicates an extremely strong positive linear relationship between the values measured by the FD-FLIM system ( $\tau_{AVG-FDFLIM}$ , vertical axis) and the TCSPC spectrometer ( $\tau_{AVG-TCSPC}$ , horizontal axis), as illustrated in **Fig. S1**.



**Fig. S1.** Scatter plot showing the correlation between the average lifetimes measured by the FD-FLIM system ( $\tau_{AVG-FDFLIM}$ ) and the TCSPC system ( $\tau_{AVG-TCSPC}$ ). The correlation coefficient ( $\rho$ ) and p-value demonstrate a strong association between the measurements of both systems.

The Bland-Altman plot shown in **Fig. S2** also demonstrates a strong agreement between the average lifetimes measured by FD-FLIM ( $\tau_{AVG-FDFLIM}$ ) and the calibrated TCSPC spectrometer ( $\tau_{AVG-TCSPC}$ ). The mean difference (bias) was 0.072 ns, indicating minimal systematic error between the lifetimes measured by the systems. The close clustering of data points around the mean difference, along with the majority of data points falling within the limits of agreement, shows that the lifetimes measured within this range by the FD-FLIM system are consistent and reliable.



**Fig. S2.** Bland-Altman plot illustrating good agreement between the average lifetimes measured by the FD-FLIM system ( $\tau_{AVG-FDFLIM}$ ) and the calibrated TCSPC spectrometer ( $\tau_{AVG-TCSPC}$ ).