

Supplementary Information

Overcoming Photon and Spatiotemporal Sparsity in Fluorescence Lifetime Imaging with SparseFLIM

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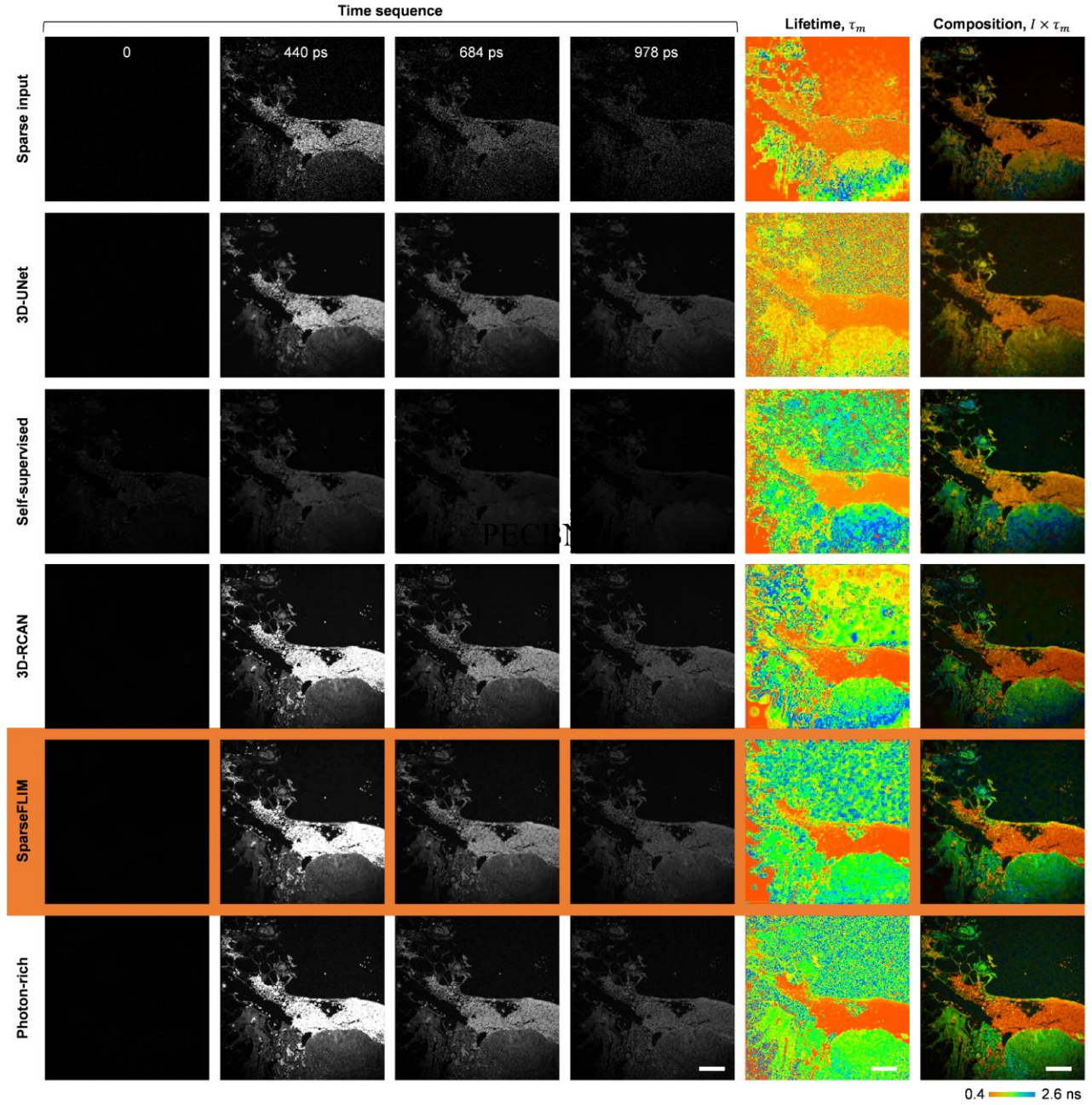
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Supplementary Note 1 Phasor approach for component decomposition

We leveraged a fit-free phasor technique¹ to characterize components and discriminate between red blood cells (RBCs) and dermal structures, rather than only determining lifetime values or decay components through biexponential fitting. Unlike fitting approaches that rely on model assumptions and can suffer from convergence issues or local optima entrapment, phasor analysis directly transforms time-resolved data into a graphical distribution providing intuitive readouts of protein-bound and free fluorophore fractions².

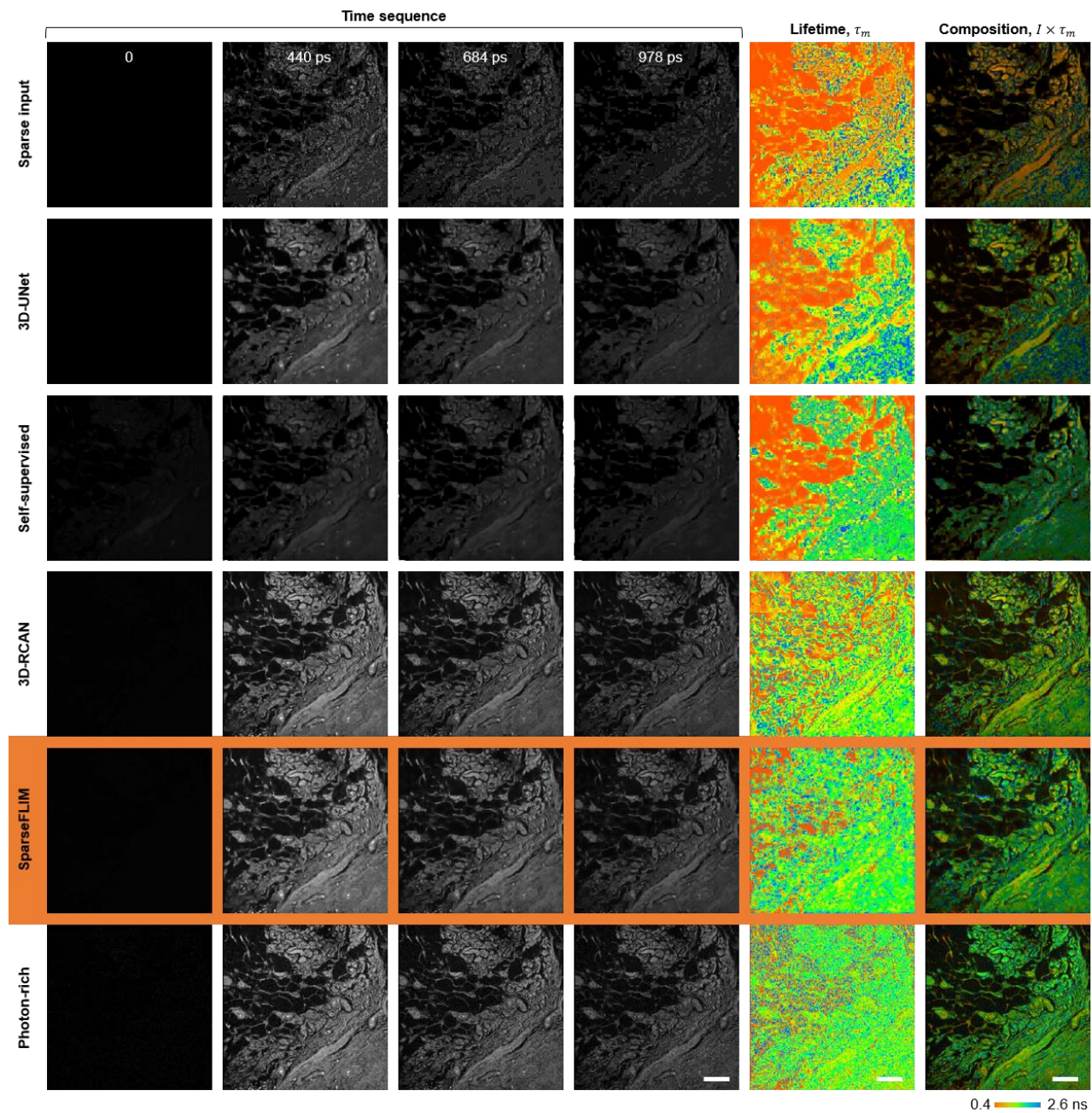
Specifically, we generated phasor plots corresponding to the Fourier transforms of the raw sparse FLIM input, deep learning reconstructed output, and sufficient photon reference data (Supplementary Fig. 5). We compared these phasor distributions and their ability to resolve cell types based on component makeup using this fit-free technique without any biased or non-linear fitting procedures. The phasor patterns following deep learning reconstruction closely aligned with the phasor transforms of the sufficient photon acquisition. The strong matching highlights the capability of the network to substantially enhance the correlation and accuracy in a completely agnostic and assumption-free manner. This consistency extended across both data interpretations based on fitting routines and this alternative fit-free method.

Additionally, we noted the noise-only component at long lifetime fractions was effectively suppressed in the network outputs (Supplementary Fig. 5c,e). This observation indicates additional denoising capabilities where the network learns to remove non-informative signal unrelated to fluorescent species of interest. The suppression enhances contrast and further improves analysis compared to the raw sparse patterns full of noise and artifacts. Thus, adopting an orthogonal phasor approach reinforced the ability of the network to not only recover accurate mean lifetime values through exponential fitting, but more broadly reconstruct reliable lifetime distributions, fractions, and contrast consistent with sufficient acquisition. This expands the applicability and underscores the robustness of the method across analysis modalities.



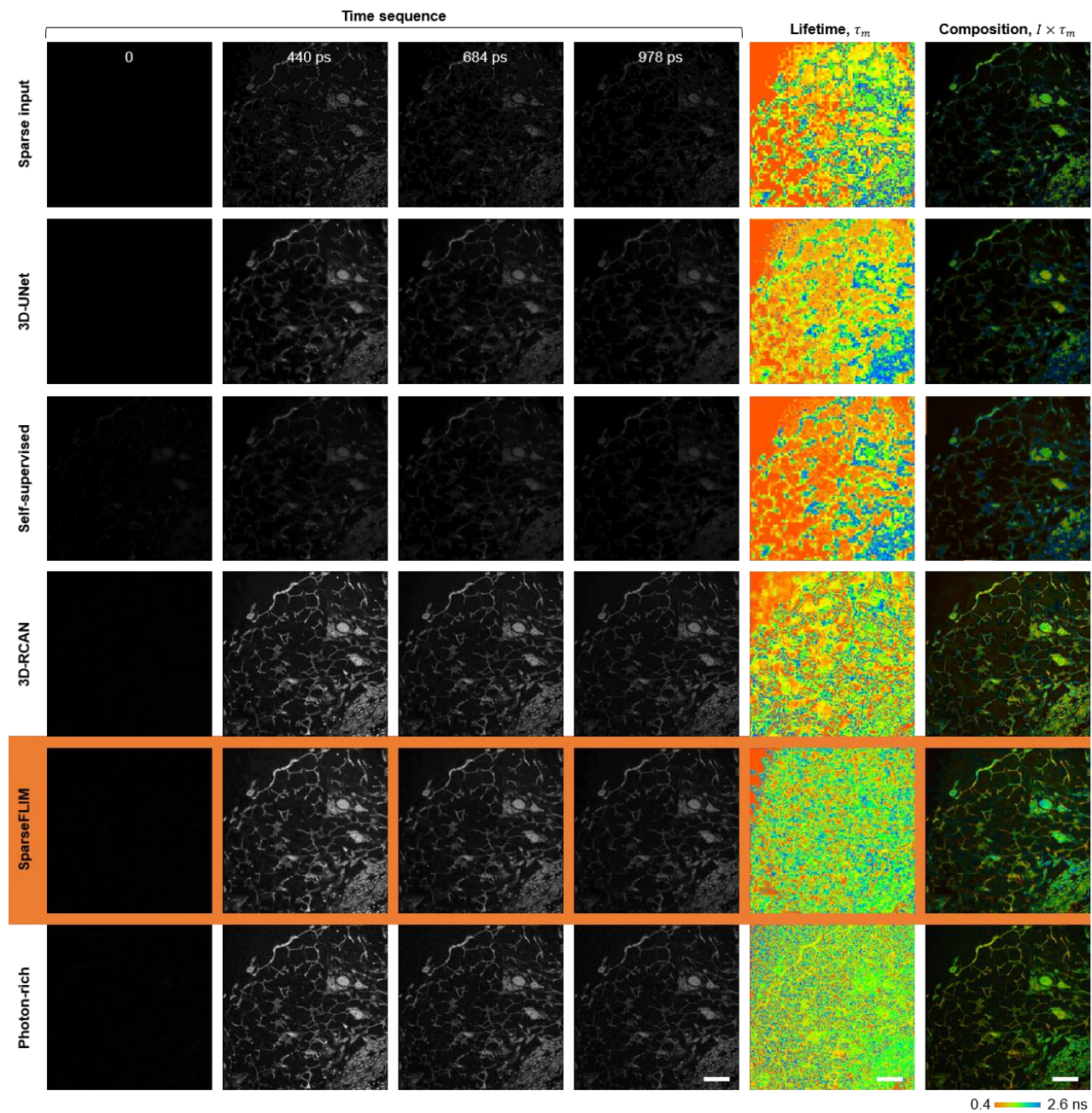
Supplementary Fig. 1 Comparative analysis of RBCs reconstruction by different models.

These models³⁻⁸ were trained with the same dataset. The gray images represent two-photon intensity at specific time points. The lifetime maps were presented in mean τ_m . The FLIM images were composited by the intensity and lifetime map. Scale bars, 100 μm .



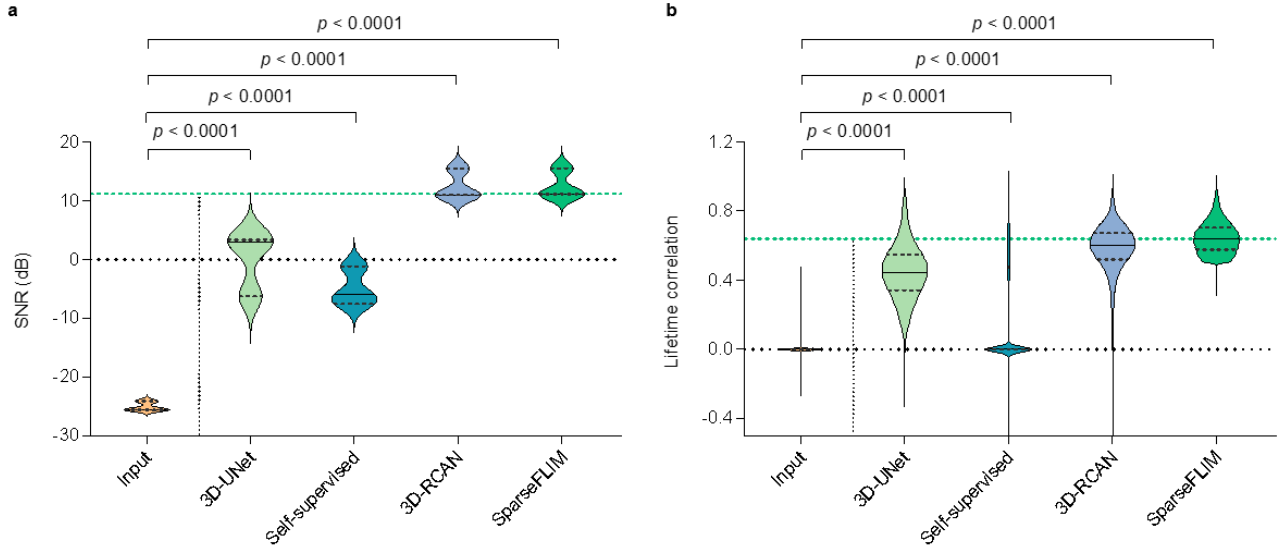
Supplementary Fig. 2 Comparative analysis of dermis reconstruction by different models.

The gray images represent two-photon intensity at specific time points. The lifetime maps were presented in mean τ_m . The FLIM images were composited by the intensity and lifetime map. Scale bars, 100 μm .



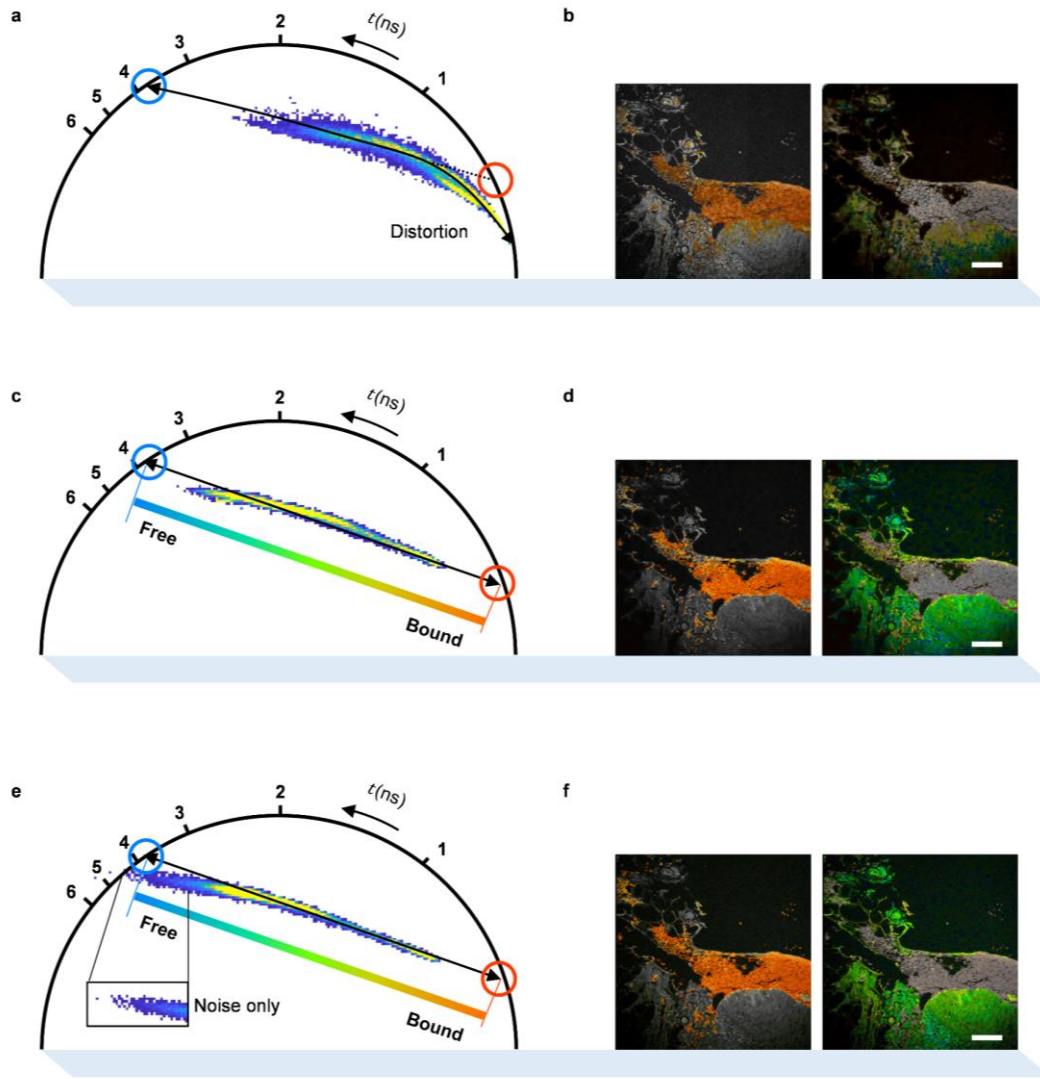
Supplementary Fig. 3 Comparative analysis of subcutaneous fat area reconstruction by different models.

The gray images represent two-photon intensity at specific time points. The lifetime maps were presented in mean τ_m . The FLIM images were composited by the intensity and lifetime map. Scale bars, 100 μm .



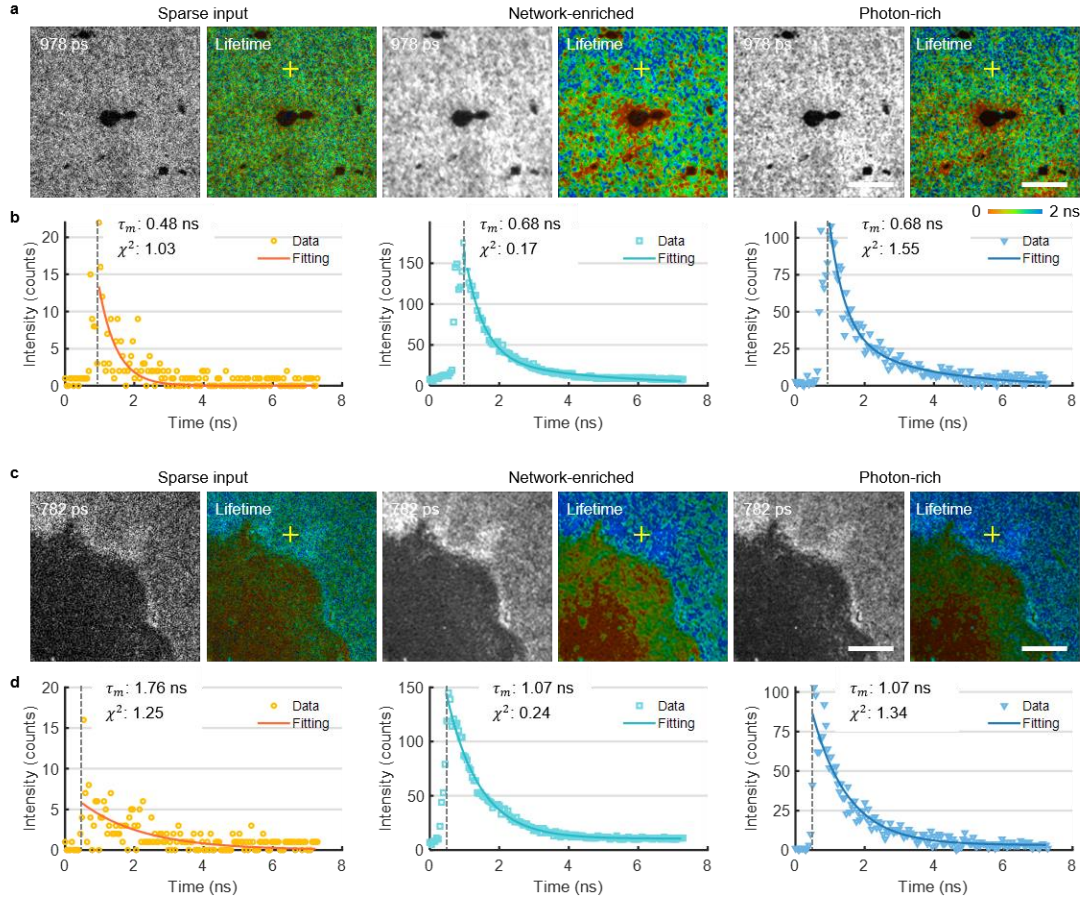
Supplementary Fig. 4 SNR and lifetime correlation comparisons among various networks.

Violin plot showing the 3D SNR (**a**) and temporal correlation (**b**) of the FLIM data before and after different network reconstructions. Two-tailed Wilcoxon matched-pairs signed rank tests were applied between the input and networks output. $n = 78,662$.



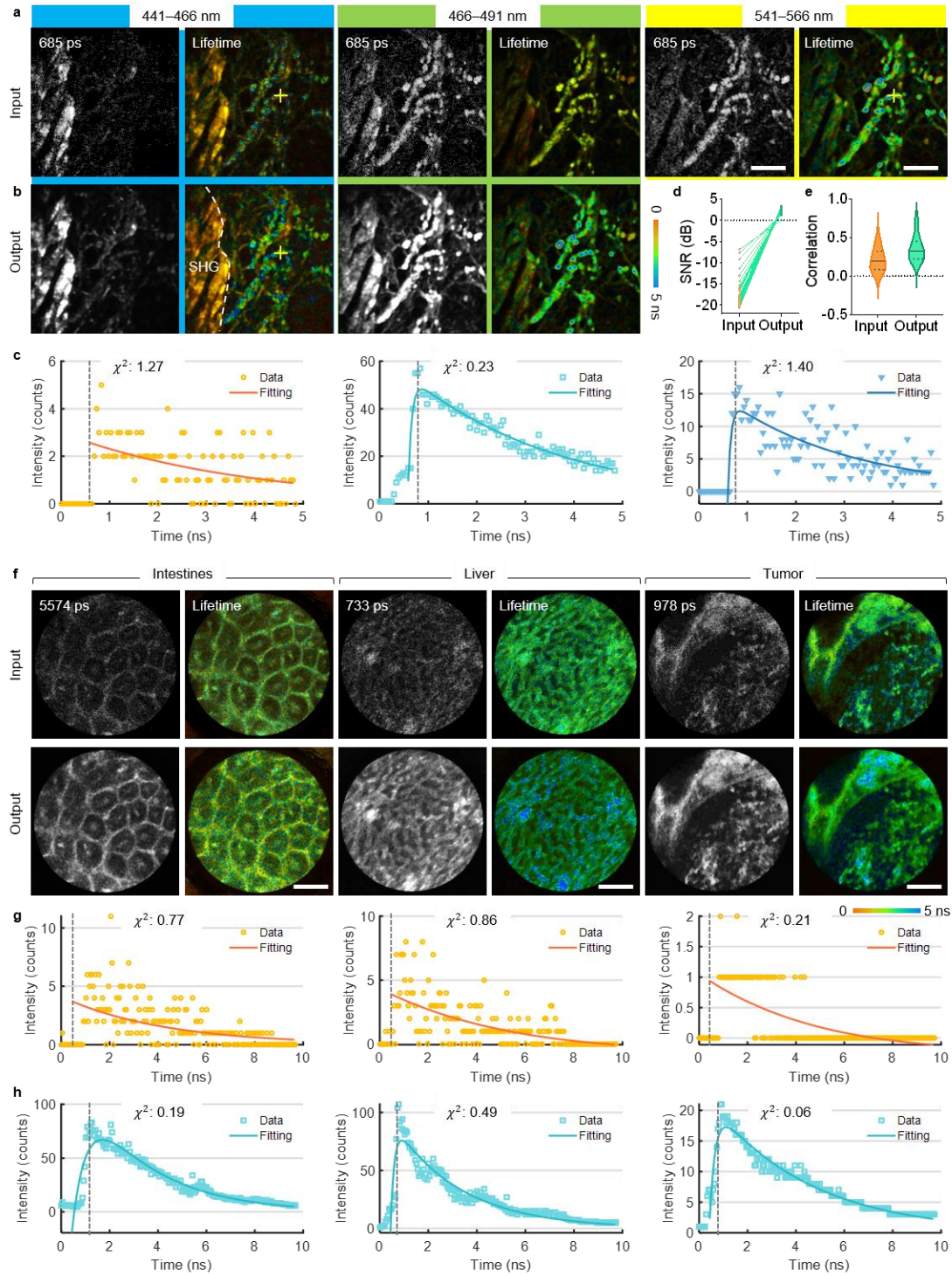
Supplementary Fig. 5 Phasor analysis of human skin tissue.

a, Phasor plot of the raw input data, with non-collinear blue (long lifetime) and orange (short lifetime) symbols representing the component decomposition. **b**, Pseudocolor images showing different lifetime species. **c**, Phasor plot of the network output data, with collinear blue (long lifetime) and orange (short lifetime) symbols representing the bicomponent decomposition. **d**, Pseudocolor images showing different lifetime species that highly match the photon-rich reference. **e**, Phasor plot of the photon-rich data, with collinear blue (long lifetime) and orange (short lifetime) symbols representing the bicomponent decomposition, and the close-up indicates the noise-only component. **f**, Pseudocolor images showing different lifetime species. Scale bar, 100 μm .



Supplementary Fig. 6 Network validation on single-photon FLIM.

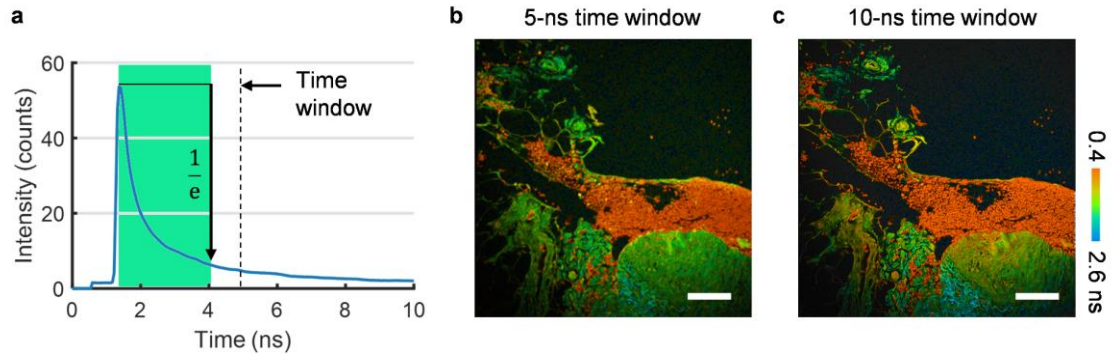
a, Single-photon FLIM images of normal liver tissue of mouse². **b**, Fluorescence intensity decay over time for the sparse photon, the network-enriched output, and the sufficient photon reference data. **c**, Single-photon FLIM images of liver tissue with boundary between non- and metastasis. **d**, Fluorescence intensity decay over time for the sparse photon, the network-enriched output, and the sufficient photon reference data. Scale bar, 200 μ m.



Supplementary Fig. 7 Network generalization performance verification.

a, Multispectral FLIM images of human skin tissue at different spectral segments. **b**, Reconstruction results of SparseFLIM. **c**, Comparison of fluorescence decay at the location indicated by the cross in **a** and **b** for the sparse photon input (441–466 nm), network output, and photon-rich acquisition (541–566 nm). **d**, Increases of the SNR. Each line represents 1 of 87 images. **e**, Violin plot showing the Pearson correlations of fluorescence decay traces before and after network inference. $n = 65,536$. Traces in the 541–566 nm channel were used as the reference for correlation calculation. **f**, Intravital endoscopic FLIM images of mouse intestines, liver, and

tumor tissues. Upper, images acquired by the lifetime endoscope. Bottom, the corresponding network-enhanced images. The fluorescence decay at the middle position of the images in **f** for the sparse photon input and network output were compared in **g** and **h**. Scale bars, 50 μm in **a** and 100 μm in **f**.



Supplementary Fig. 8 Comparative analysis of lifetime estimates with different time windows.

a, Fluorescence intensity decay at a full time window of 10 ns for the sufficient photon reference data (cumulative from 262k pixels). The main fluorescence lifetime of endogenous FAD is located in the green region, and the dashed line indicates the time window for autofluorescence lifetime estimation in the experiments. Lifetime map reconstructed by fitting the fluorescence decays within a 5 ns temporal window (**b**) and 10-ns window (**c**). Scale bars, 100 μm .

Supplementary Table 1 Comparative analysis of various key frame selection methods

Model	SNR (dB)	SSIM	Correlation
Input	-5.1 $\pm$ 6.7	0.4 $\pm$ 0.1	0.6 $\pm$ 0.3
BasicVSR++ ⁹	10.1 $\pm$ 1.6	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2
BasicVSR ¹⁰	11.1 $\pm$ 1.9	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2
Key frame selection For SparseFLIM			
[]	11.1 $\pm$ 2.0	0.8 $\pm$ 0.1	0.8 $\pm$ 0.3
[10]	11.2 $\pm$ 1.8	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2
[8, 10]	11.4 $\pm$ 2.0	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2
[8, 10, 16]	11.7 $\pm$ 1.9	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2
[8, 10, 12, 16]	11.8 $\pm$ 2.0	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2
[8, 10, 12, 16, 20]	11.5 $\pm$ 1.9	0.8 $\pm$ 0.1	0.8 $\pm$ 0.2

Supplementary references

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