Electronic Supplementary Information

A Colorful Approach Towards Developing New Nano-based Imaging Contrast Agents for Improved Cancer Detection

Helen R. Salinas[1,†], Dominie L. Miyasato[1,†], Olga E. Eremina[1, †], Rodolfo Perez[1], Karen L. Gonzalez[1], Alexander T. Czaja[1], Sean Burkitt[1], Arjun Aron[1], Augusta Fernando[1], Lauro S. Ojeda[1], Kimberly Larson[1], Ahmed W. Mohamed[1], Jos L. Campbell[1], Beth A. Goins[2], Cristina Zavaleta [1,*]

[1] Department of Biomedical Engineering, University of Southern California, 1002 Child's Way, Los Angeles, CA 90089, United States.
[2]Department of Radiology, University of Texas Health Science Center, 7703 Floyd Curl Dr. San Antonio, TX 78229, United States.

* Correspondence: Cristina Zavaleta, czavalet@usc.edu; Tel.: +01-213-764-7999
† These authors contributed equally to this work.

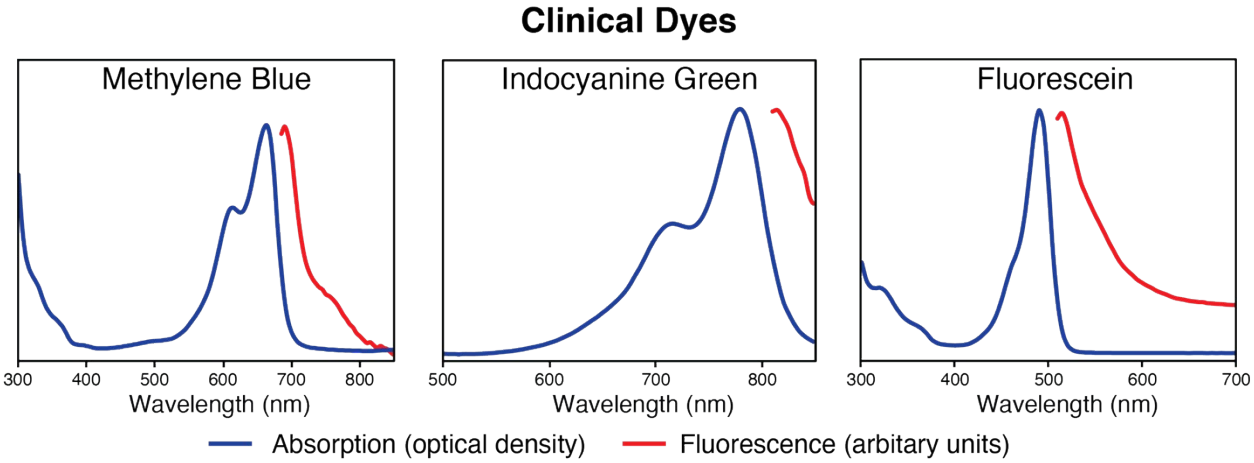


Figure S1. Absorption and fluorescence spectra of clinical dyes (Methylene Blue in H_2O; Indocyanine Green in DMSO; Fluorescein in 0.5 M NaOH).

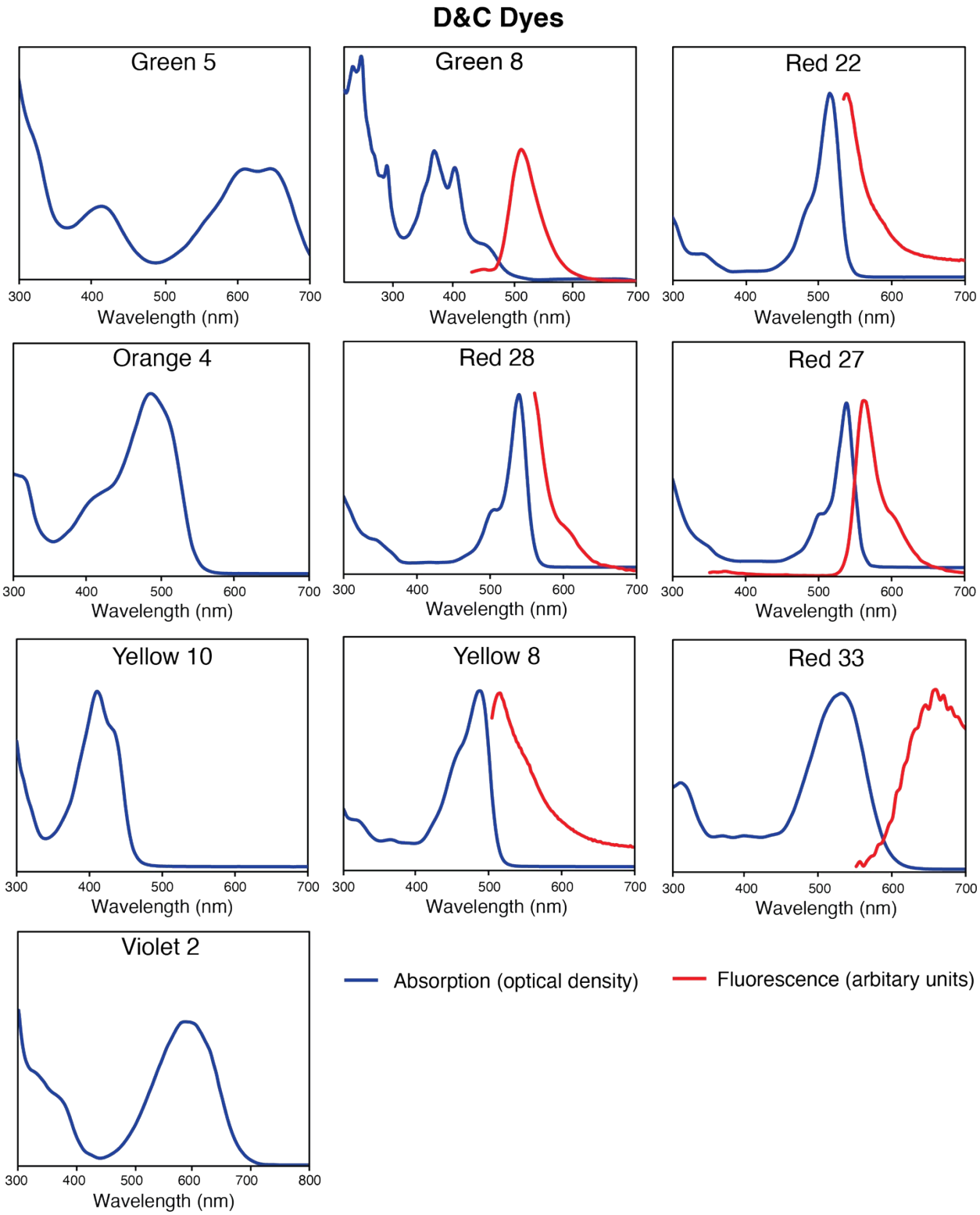


Figure S2. Absorption and fluorescence spectra of D&C dyes (Green 5, Green 8, Red 22, Orange 4, Red 28, Yellow 10, and Red 33 in H_2O; Red 27 and Violet 2 in DMSO).

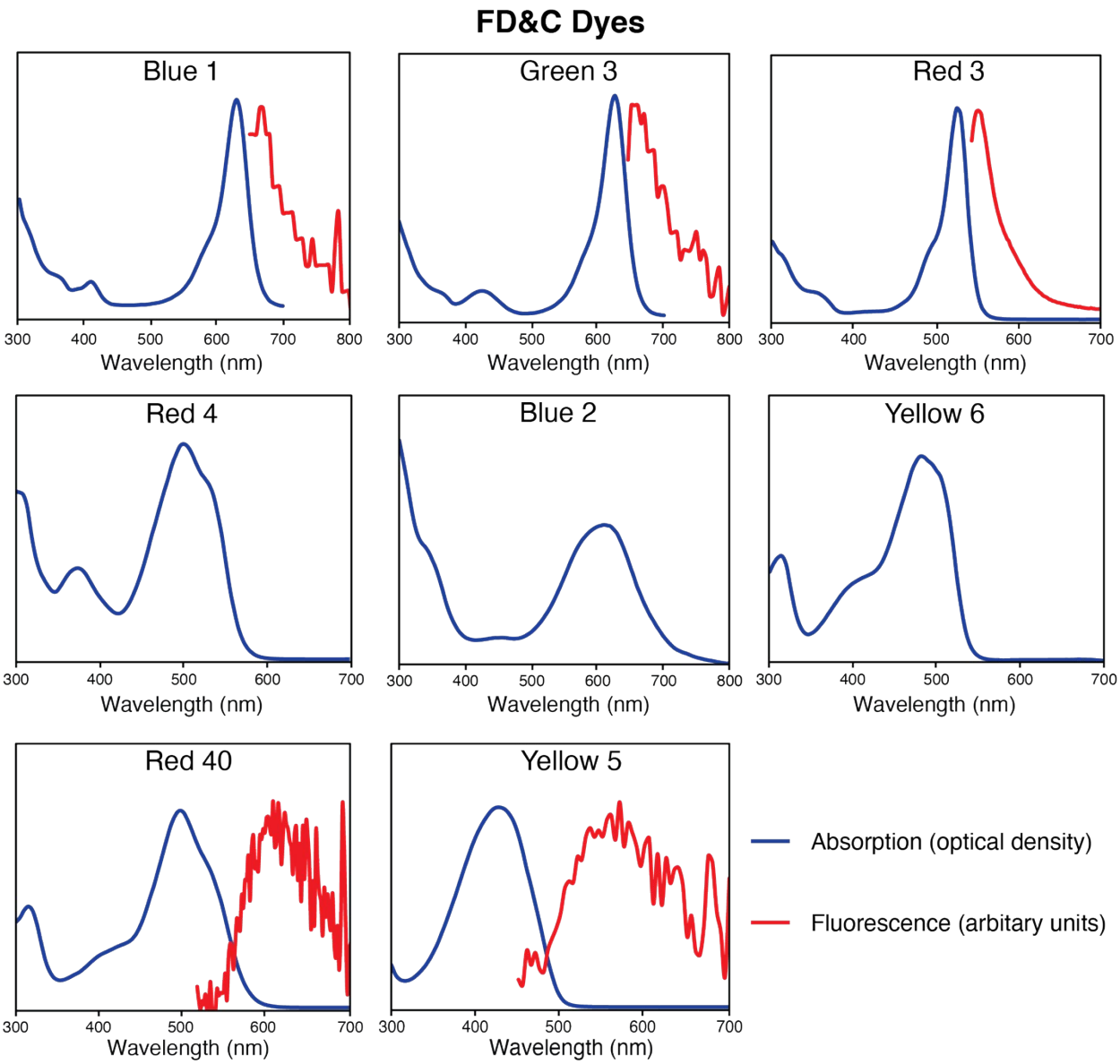


Figure S3. Absorption and fluorescence spectra of FD&C dyes (Blue 1, Red 3, Red 4, Yellow 6, Red 40, and Yellow 5 in H_2O; Blue 2 in DMSO).

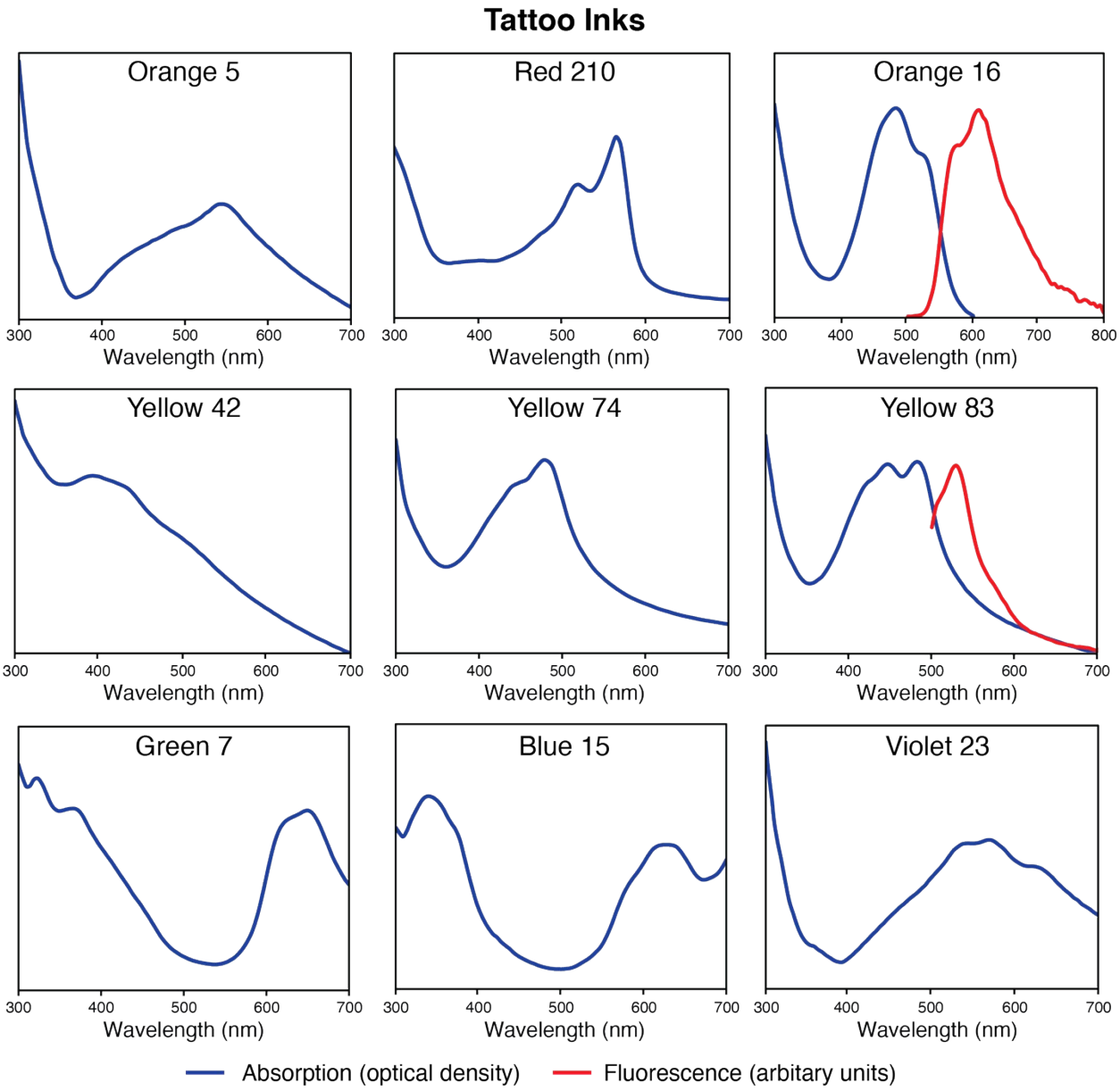


Figure S4. Absorption and fluorescence spectra of tattoo inks (all tattoo inks were dissolved in H_2O).

Furthermore, fluorescence efficiency for the best-performing dyes was compared in PBS solution with pH 7.4. As some of the clinical dyes (Fluorescein and Indocyanine Green) and Red 27 are insoluble in water, initial solution of Fluorescein was prepared in 0.1 M NaOH; Red 27 and Indocyanine Green – in DMSO, and then these solutions were diluted at least 10 times with PBS.

Quantum yield, i.e. the efficiency of converting absorbed light into emitted light, for each dye was calculated by the most frequently and most reliable comparative method described by Williams et al.,[1] which involves the use of well characterized standard samples with known φ-values. Fluorescein solution was chosen as a standard sample.[2]

$$\varphi = \varphi_{FL} \cdot \frac{F}{F_{FL}} \cdot \frac{A_{FL}}{A} \cdot \left(\frac{n_{FL}}{n}\right)^2$$

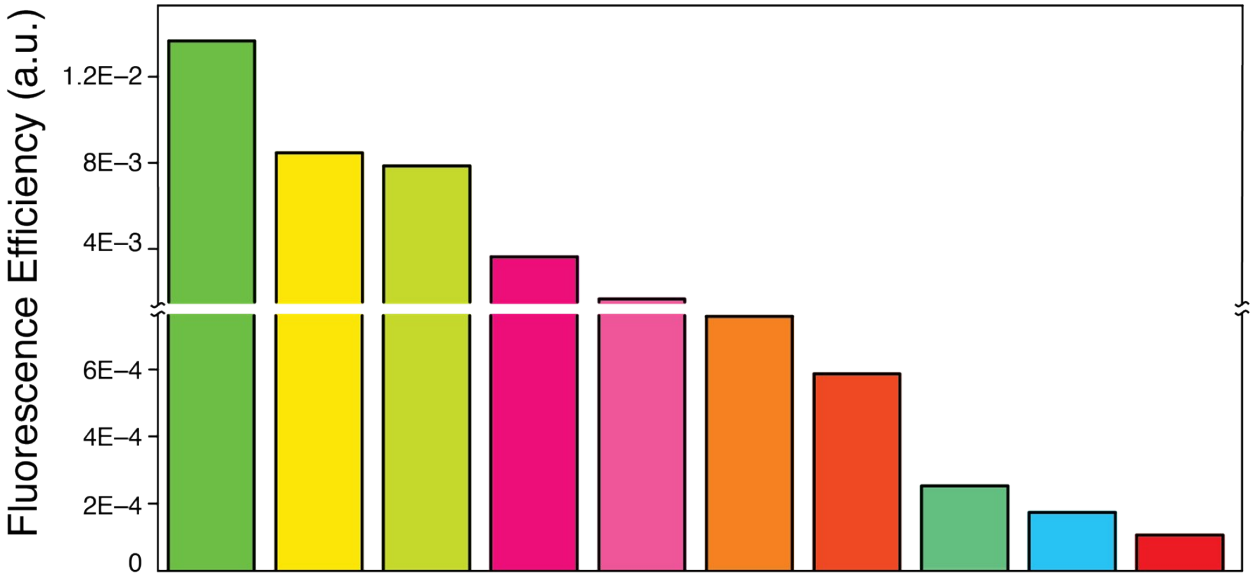
where φ and φ_{FL} – quantum yields for the investigated dye and fluorescein, respectively;
F and F_{FL} – fluorescence intensities for the investigated dye and fluorescein, respectively;
A and A_{FL} – absorbance at excitation wavelength for the investigated dye and fluorescein, respectively;
n and n_{FL} – refractive index of the solvent used for the investigated dye and fluorescein solution, respectively (n_{H2O} = 1.333 and n_{EtOH} = 1.36).

Table S1. Fluorescence properties for the best-performing investigated dyes in different media.

Dye	Solvent	λ_{ex}, nm	λ_{em}, nm	Quantum yield
Fluorescein (FL)	EtOH	485	521	0.95[2]
	PBS	485	515	0.86
	1 M NaOH	485	515	0.92[3]
Red 22 (R22)	PBS	515	538	0.63
	H_2O	515	538	0.68
Red 28 (R28)	PBS	540	600	0.53
	H_2O	540	600	0.41
Red 3 (R3)	PBS	525	549	0.08
	H_2O	525	549	0.07
Red 27 (R27)	PBS	540	610	0.15
	EtOH	555	620	0.42
	H_2O	540	610	0.15
Green 8 (G8)	PBS	455	515	0.34
	0.5 M NaOH	455	515	0.35
Orange 16 (O16)	PBS	480	617	0.014
	H_2O	480	617	0.016
Methylene Blue (MB)	PBS	600	685	0.016
	CH_2Cl_2	664	690	0.52[4]
Indocyanine Green (ICG)	PBS	785	815	0.027[5]
	EtOH/H_2O	785	815	0.084[5]

References:

1. A. T. R. Williams, S. A. Winfield and J. N. Miller, *Analyst*, 1983, **108**, 1067-1071.
2. X.-F. Zhang, J. Zhang and Limin Liu, *J Fluoresc*, 2014, **24**, 819-826.
3. D. Magde, R. Wong and P. G. Seybold, *Photochem Photobiol*, 2002, **75**, 327-334.
4. M. Mirenda, C. A. Strassert, L. E. Dicelio and E. S. Román, *ACS Appl Mater Interfaces*, 2010, **2**, 1556-1560.
5. T. J. Russin, E. İ. Altınoğlu, J. H. Adair and P. C. Eklund, *J Phys Condens Matter*, 2010, **22**, 334217.



Dye	G8	Y8	*FL	R27	R22	O16	R28	*ICG	*MB	R3
λ_{ex} (nm)	405	465	465	570	500	405	500	745	640	500
λ_{em} (nm)	530	530	530	610	550	630	550	810	690	550
c (mg/mL)	12.5	0.0625	0.156	43.75	0.120	35	0.11	0.78	0.01	0.391
Media	0.5 M NaOH	H_2O	0.5 M NaOH	DMSO	H_2O	H_2O	H_2O	DMSO	H_2O	H_2O

Fig. S5. Fluorescence efficiency of the best-performing dyes and pigments using their optimal concentrations, solvent, and filter settings to evaluate maximum fluorescence potential (*denotes the clinically approved dyes). Fluorescence efficiency is defined here as the amount of fluorescence emission signal divided by the incident fluorescence excitation signal (theoretical maximum ratio of one and is therefore unitless).

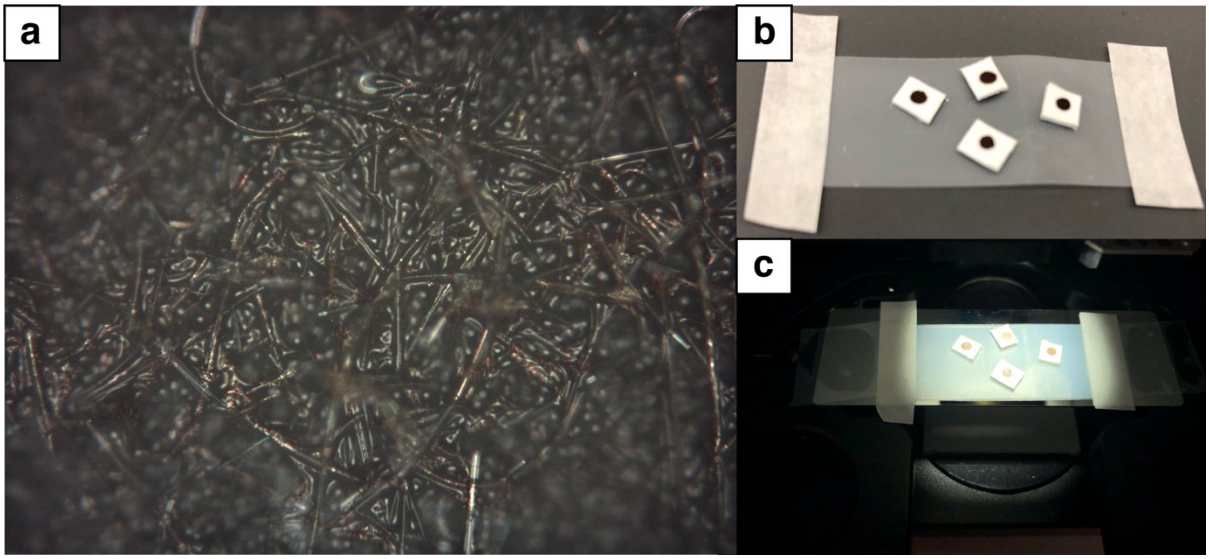


Figure S6. (a) Microphotograph of quartz paper with 10 nM AuNPs colloid before SERS measurements; (b) photograps of quartz paper before applying Green 8 or Orange 16 solution on the surface and (c) after applying Green 8 or Orange 16 solution before the SERS measurement.

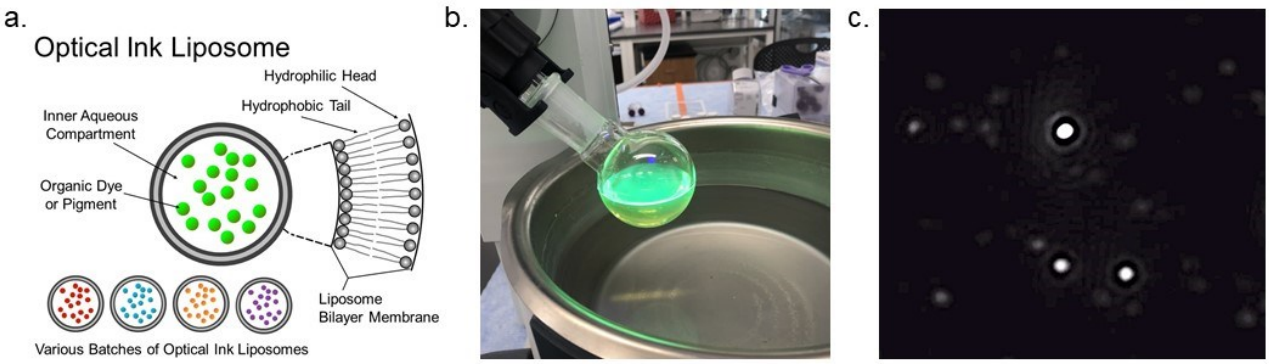


Figure S7. (a) Nanoparticle schematic of our liposomes; (b) Fluorescence generation from FDA approved drug and cosmetic dye G8 used in our liposomal nanoparticle formulation; (c) Nanoparticle tracking analysis (NTA) image of our 180 nm liposomes encapsulated with G8 dye used in this the study.

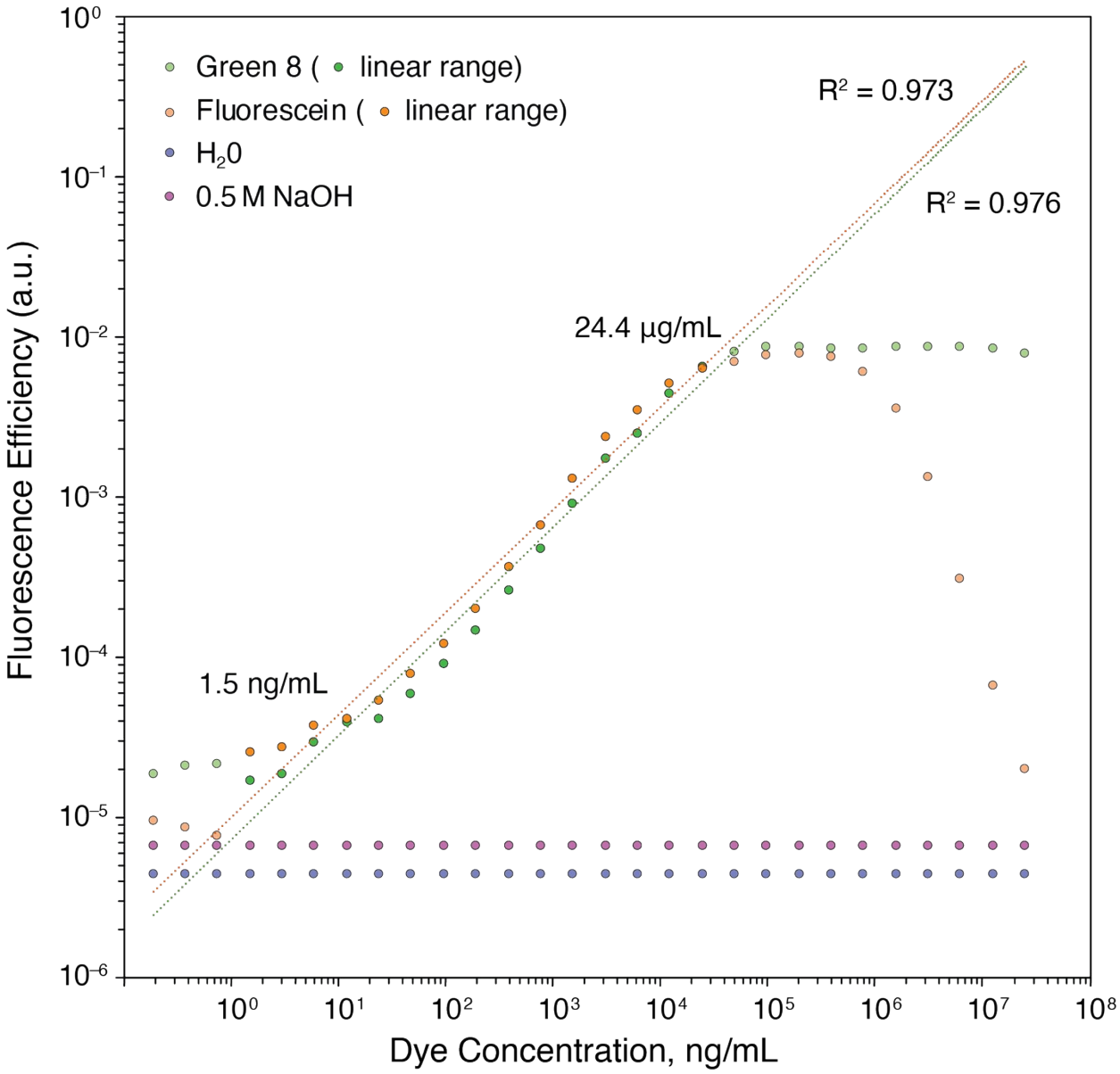


Figure S8. Linear Dynamic Range of G8 dye and fluorescein dye. Both dyes exhibit the same linear dynamic range from 1.5 ng/ml to 24.4 ug/ml. Notice how the fluorescence signal for G8 plateaus at higher concentrations and the fluorescent signal for fluorescein drops off at higher concentrations.

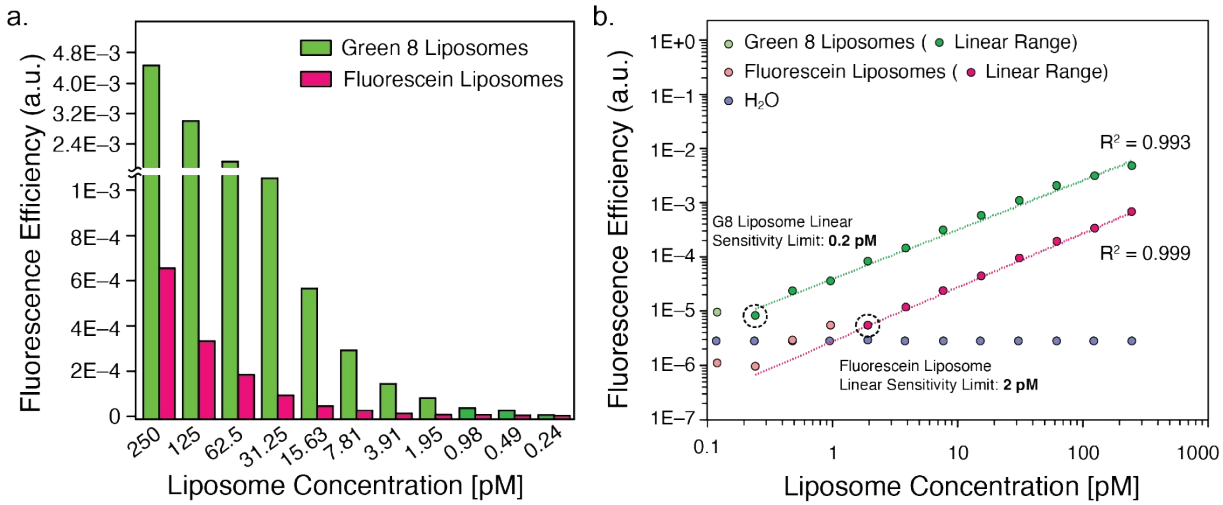


Figure S9. Fluorescence sensitivity of G8-liposomes and FL-liposomes. (a) Fluorescence efficieny of G8-liposomes compared with FL-liposomes (b) Linear sensitivity plot for both liposome batches. Notice the linear sensitivity limit of G8 dye is 0.2 pM whereas the linear sensitivity limit for FL-liposomes is 2 pM. Both dyes exhibit strong fluorecent signal for imaging, but the G8-liposomes are more sensitive.

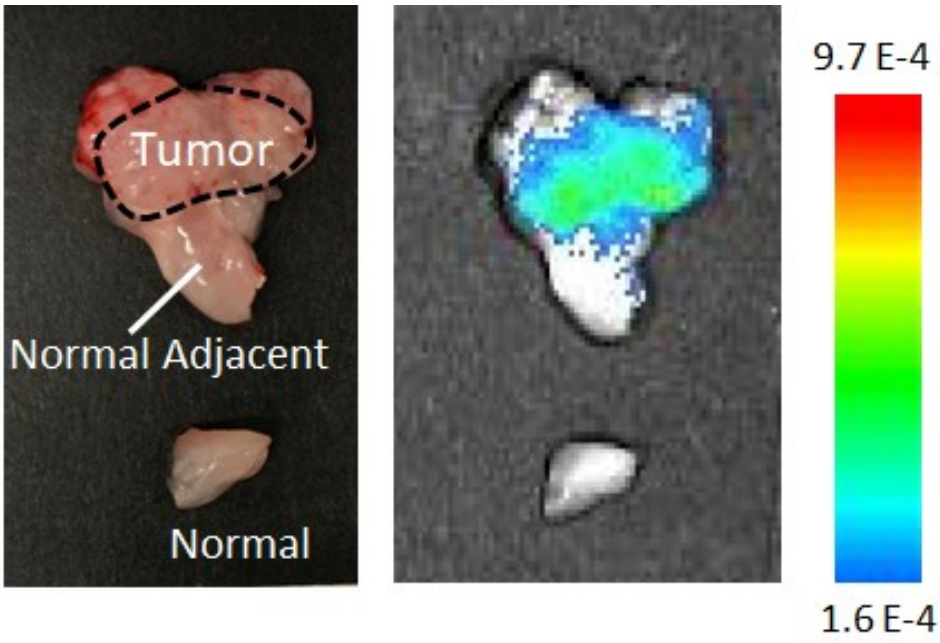


Figure S10. LS174T tumor retaining 180 nm liposomes surrounded by healthy adjacent tissue. Color bars represents fluorescence efficiency which is defined here as the amount of fluorescence emission signal divided by the incident fluorescence excitation signal. So the maximum theoretical number would be 1 and is therefore unitless.

Table S2.

Figure 5	Tumor to Adj. Tissue Ratio	Tumor to Control Ratio
LS174T	22.46	17.25
HeLa	22.08	17.00

Figure 6	Tumor to Adj. Tissue Ratio	Tumor to Control Ratio
180 nm	4.00	9.41
360 nm	4.39	1.56

Figure 7	Tumor to Adj. Tissue Ratio	Tumor to Control Ratio
O16 Liposomes	8.15	4.91
O16 Free Dye	2.62	1.12

Video S1. Live surgical resection of fluorescent LS174T tumor from adjacent normal tissue (dark background). Mouse was IV injected with G8-liposomes and euthanized 4 hr. post injection. Notice how the adjacent tissue does not show significant autofluorescence as compared to the neighboring tumor tissue, and the tumor is easily visualized with fluorescence image guidance.

Video S2. Intravital multiphoton imaging of our newly fabricated G8-liposomes flowing through the vasculature of a living mouse in real-time at 30 frames per second.