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Silicon-RosIndolizine fluorophores with shortwave infrared absorption and emission profiles enable in vivo fluorescence imaging

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In vivo fluorescence imaging in the shortwave infrared (SWIR, 1,000–1,700 nm) and extended SWIR (ESWIR, 1,700–2,700 nm) regions has tremendous potential for diagnostic imaging. Although image contrast has been shown to improve as longer wavelengths are accessed, the design and synthesis of organic fluorophores that emit in these regions is challenging. Here we synthesize a series of silicon-RosIndolizine (SiRos) fluorophores that exhibit peak emission wavelengths from 1,300–1,700 nm and emission onsets of 1,800–2,200 nm. We characterize the fluorophores photophysically (both steady-state and time-resolved), electrochemically and computationally using time-dependent density functional theory. Using two of the fluorophores (SiRos1300 and SiRos1550), we formulate nanoemulsions and use them for general systemic circulatory SWIR fluorescence imaging of the cardiovascular system in mice. These studies resulted in high-resolution SWIR images with well-defined vasculature visible throughout the entire circulatory system. This SiRos scaffold establishes design principles for generating long-wavelength emitting SWIR and ESWIR fluorophores.

Biomedical imaging is an invaluable tool in the hands of research scientists and medical professionals. It allows for the visualization of internal structures, diagnosis of diseases and injuries, and provides spatial information on bodily regions of interest. Of the types of imaging modalities currently available, shortwave infrared (SWIR, 1,000–1,700 nm; sometimes referred to as the second and third near-infrared regions; NIR-II and NIR-III, respectively)^{1–3} fluorescence imaging offers several advantages over traditional forms of in vivo imaging in that it avoids harmful ionizing radiation, can provide high spatiotemporal resolution, enables targeted imaging of specific diseases/regions and provides rapid data acquisition^{4–9}. Magnetic and

high-energy radiation techniques underperform at one or more of these desirable capabilities^{4,5,10}. In this way, SWIR fluorescence imaging has the capacity to be a specific, rapid, non-toxic and high-resolution technique for in vivo imaging with promising biomedical applications. Improvements to organic dye properties are needed to realize this type of imaging, especially with regard to accessing longer wavelengths.

Compared with visible light, light in the SWIR and extended SWIR (ESWIR 1,700–2,700 nm, including the rarely accessed NIR-IV region, in which the total attenuation coefficients of tissues are smallest)^{11–13} regions offers several advantages for full body in vivo imaging by limiting light scattering, absorption and the autofluorescence of

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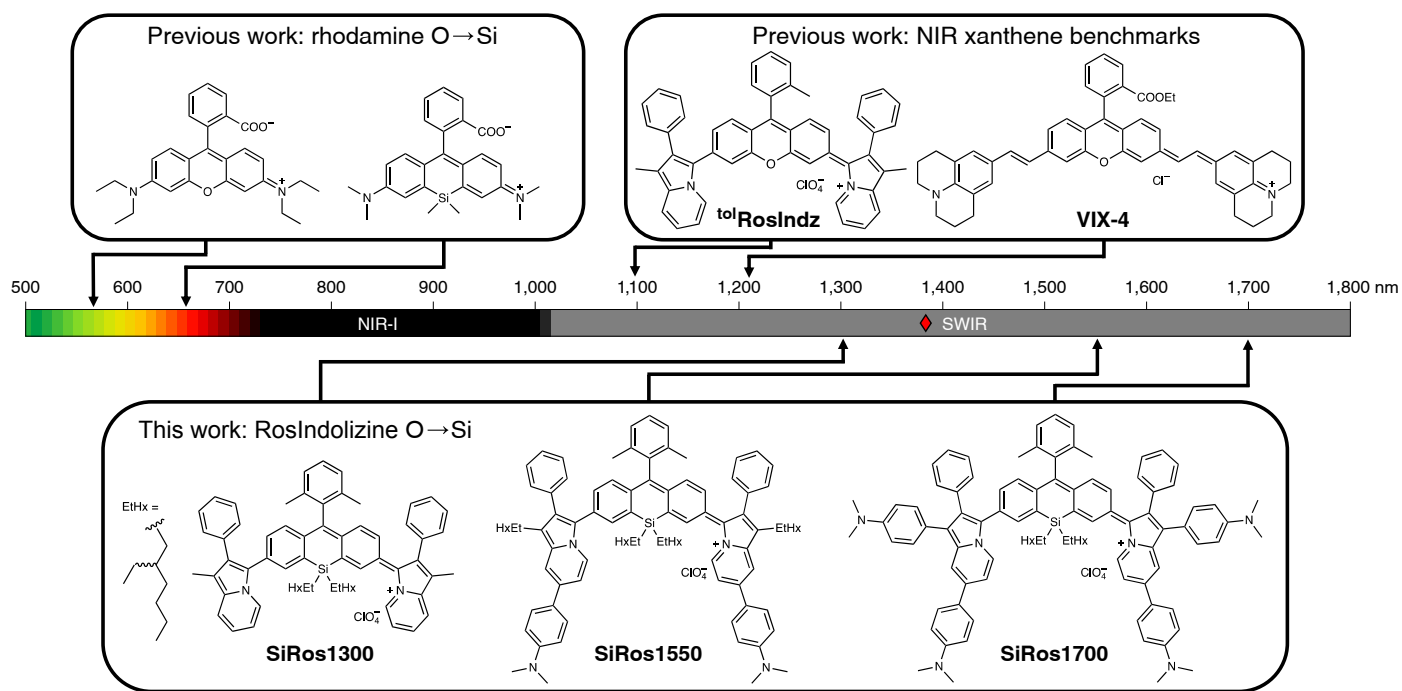


Fig. 1 Emission maxima of xanthene and silicon-substituted xanthene-based fluorophores. Structure of xanthene and silicon-substituted xanthene-based fluorophores with alkyl amine donors from the literature (top-left box), xanthene-based fluorophores with heterocyclic donors from the literature (top-

right box) and SiRos fluorophores presented herein (bottom box). Respective emission maxima are indicated by arrows. The small-molecule fluorophore with longest wavelength emission maxima reported so far is shown as a red diamond on the scale as a benchmark²⁹.

biological tissues⁴. These factors impede excitation light penetration and the contrast of the image obtained¹⁴. Although PbS quantum dots and cerium-based materials have been shown to exhibit peak emission past 1,400 nm, these materials are toxic, making them undesirable for in vivo applications^{15,16}. To the best of our knowledge, there are no organic small-molecule fluorophores with peak emission beyond 1,400 nm, despite the great potential for increased image quality. Weak tail emissions are currently used to generate images beyond 1,400 nm from organic small molecules^{17–19}.

Organic fluorophores are extremely diverse with a range of classes offering tunable spectral properties. Although there is an abundance of organic fluorophores that absorb and emit in the NIR (700–1,000 nm), there are still only a handful that absorb and emit in the SWIR. These SWIR organic fluorophores have provided the highest resolution in vivo fluorescence images so far. Until recently, xanthene-type fluorophores such as fluorescein and rhodamine were generally considered visible-region fluorophores²⁰. A SWIR-peak-emitting xanthene-based fluorophore was recently synthesized by installing indolizine heterocycles in place of alkyl amine donors, resulting in a ~400 nm (1.01 eV) bathochromic shift in the absorbance of the material to 930 nm and a SWIR emission maximum at 1,092 nm (ref. 21). Soon thereafter, *para*-functionalized styrene-based donors were used to yield a set of fluorophores with remarkably low-energy emission maxima at 1,210 nm (ref. 9). These molecules were observed to yield rapid, high-resolution in vivo SWIR fluorescence images of vascular tissues via encapsulation of the fluorophores in a micelle. This approach has led to several reports on SWIR-emitting xanthene-type fluorophores, including the discovery of a 1,256-nm-emitting fluorophore made possible by indolizine-donor-group tuning via the addition of *N,N*-dimethylaniline (DMA) groups to the heterocycle periphery^{22,23}. Modification of the xanthene core by replacing oxygen with silicon is known to induce bathochromic shifts in peak emission by ~90 nm (0.34 eV; Fig. 1)^{24–28}. Here we investigate the combination of a silicon-substituted xanthene

core with DMA-decorated indolizine donors to access longer wavelengths for absorption and emission. Through this approach we generate long-wavelength emitting small-molecule organic fluorophores that extend >300 nm beyond the current state of the art, with emission onsets (defined as 5% maximum emission intensity value) extending into the ESWIR region (Fig. 1)^{9,29,30}.

Results and discussion

Fluorophore design and synthesis

Synthesis of the silicon-RosIndolizine (SiRos) dyes makes use of each of the three distinct halides of 2-bromo-4-chloro-1-iodobenzene iteratively to build the SiRos core and install donor functionality (Fig. 2). Alcohol **1** is synthesized as previously reported from 2-bromo-4-chloro-1-iodobenzene and 2-bromo-4-chlorobenzaldehyde³¹; **1** is subsequently deoxygenated with $\text{BF}_3/\text{SiEt}_3\text{H}$ to give diarylmethane **2** in 99% yield. Double lithium-bromide exchange with **2** is used to form a cyclized silane intermediate when reacted with dichlorobis(2-ethylhexyl)silane. This crude intermediate is oxidized with KMnO_4 to give the ketone product **3** in 37% overall yield. The 2-ethylhexyl substituent was chosen to (1) incorporate solubilizing groups to the core to aid in synthetic ease, (2) increase sterics of the fluorophore to diminish aggregation and (3) incorporate bulky groups to slow nucleophilic attack at the core as was observed in previous iterations of indolizine-xanthene fluorophores³². C–H activation of the indolizine donor groups, **4–6** (synthesized as previously reported)^{33,34}, is performed using aryl chloride **3** and a palladium catalyst to yield the silicon-substituted xanthenes, **7–9**, in 46–88% yield. The final dyes are synthesized via a one-pot reaction sequence beginning with a Grignard reaction using 2,6-dimethylphenylmagnesium bromide to yield the alcohol intermediates, followed by formation of the fluorophore via acidic work-up with 2 M HClO_4 (aq). Installation of the xylyl group via the Grignard reaction is an integral component of dye design, as larger *ortho* groups about the xanthene core have demonstrated increased

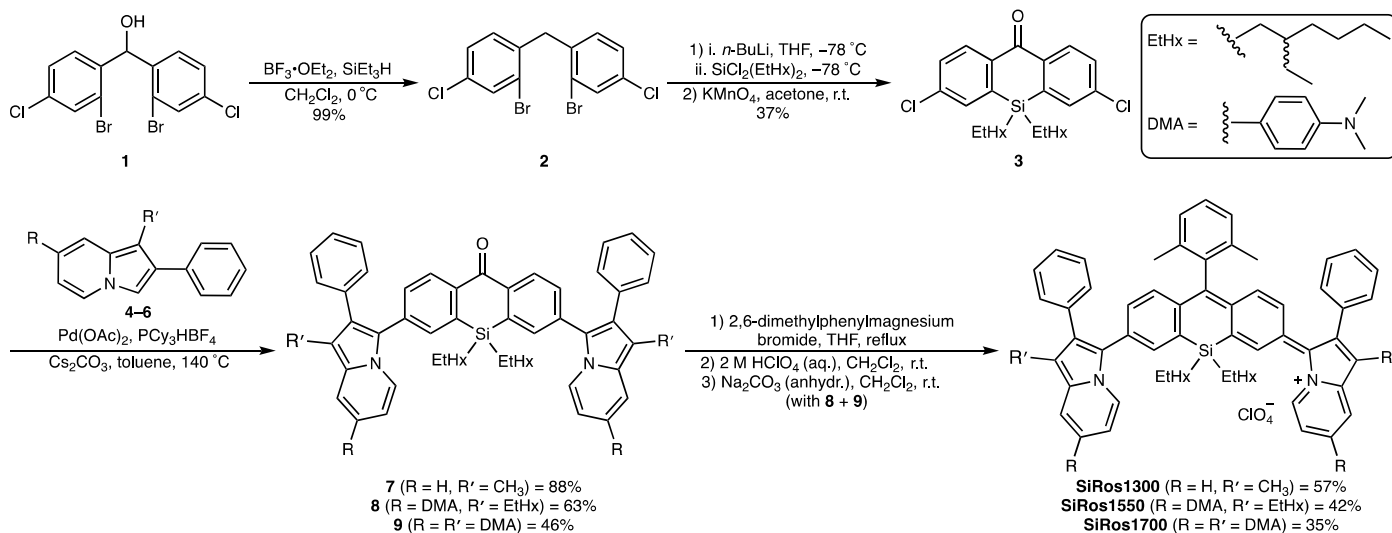


Fig. 2 | Full synthetic route to SiRos1300, SiRos1550 and SiRos1700. Synthesis of the SiRos fluorophores begins with a deoxygenation reaction followed by a silylation cyclization reaction and subsequent oxidation to yield the silicon-substituted xanthene core. A palladium-catalysed C–H activation reaction is then

used to install the respective indolizine donors. A Grignard reaction followed by an acidic work-up yields the final SiRos fluorophores as the perchlorate salt. Further work-up over anhydrous base is required for the SiRos dyes containing DMA groups. EtHx is 2-ethylhexyl abbreviated.

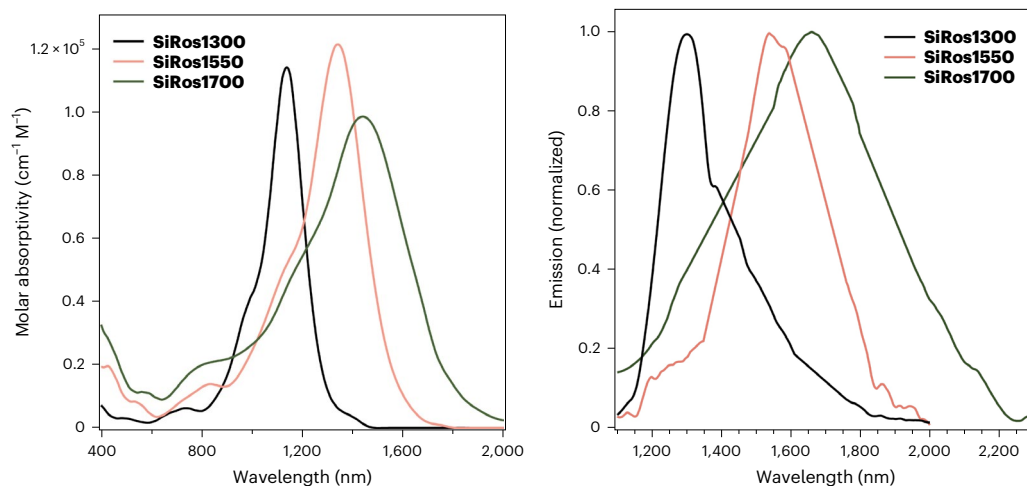


Fig. 3 | Molar absorptivity and normalized emission of SiRos1300, SiRos1550 and SiRos1700. Molar absorptivity spectra (left) and normalized and solvent-reabsorption-corrected emission spectra (right) of the fluorophores in a 1×10^{-5} M solution of CH_2Cl_2 .

stability towards hydrolytic decomposition^{22,23,32}. Although **SiRos1300** is isolable directly from the acid work-up, **SiRos1550** and **SiRos1700** require an additional biphasic reaction in dichloromethane (CH_2Cl_2) over anhydrous Na_2CO_3 to deprotonate the DMA groups. This process is monitored via absorption spectroscopy as the protonated amine derivatives demonstrate a nearly identical absorption to **SiRos1300**, and a bathochromic shift in the absorption spectrum is observed as the deprotonation reaction proceeds. With **SiRos1300**, **SiRos1550** and **SiRos1700** in hand, their photophysical properties were studied.

Steady-state absorption and emission spectroscopy

Absorption spectroscopy reveals remarkably low-energy maxima at 1,140 nm, 1,348 nm and 1,440 nm for **SiRos1300**, **SiRos1550** and **SiRos1700** in CH_2Cl_2 , respectively (Fig. 3). The exchange in the xanthene core from oxygen to silicon results in a bathochromic shift in the absorbance maxima of 0.25 eV (210 nm) from the previously reported¹⁰ RosIndz to **SiRos1300**, which is comparable with past literature reports of a bathochromic shift of 0.34 eV (90 nm) observed

for traditional rhodamine dyes with alkyl amine donors (Fig. 1)³⁵. **SiRos1550** has the largest molar absorptivity (ϵ) in CH_2Cl_2 of the dye series, at $122,000 \text{ M}^{-1} \text{ cm}^{-1}$, with **SiRos1300** and **SiRos1700** at $115,000 \text{ M}^{-1} \text{ cm}^{-1}$ and $98,000 \text{ M}^{-1} \text{ cm}^{-1}$, respectively (Table 1). As observed previously for the 1,7-DMA indolizine donor³⁴, the steric interaction between the additional DMA at the 1-position of indolizine and the phenyl ring at the 2-position lowers the molar absorptivity of **SiRos1700** to $98,000 \text{ M}^{-1} \text{ cm}^{-1}$, resulting in a broadened absorption profile compared with **SiRos1300** and **SiRos1550**. Although all dyes are nearly black when concentrated, **SiRos1300** is colourless, **SiRos1550** is orange and **SiRos1700** is green at concentrations used for absorption and emission spectroscopy ($\sim 1 \times 10^{-5} \text{ M}$) due to higher-energy visible-region absorption bands. The absorption of the dyes in acetonitrile (CH_3CN) is also studied to understand how increasing solvent polarity affects the photophysical properties of the dyes (Table 1 and Supplementary Fig. 2). For all dyes, the absorption profiles are observed to broaden and shift towards higher energy in CH_3CN , and ϵ decreases to just over half of what is observed in CH_2Cl_2 . Overall, the

Table 1 | Photophysical data of SiRos1300, SiRos1550 and SiRos1700 in the specified solvents

Dye	Solvent	$\lambda_{\text{abs}}^{\text{max}}$ (nm)	$\lambda_{\text{emis}}^{\text{max}}$ (nm)	$\lambda_{\text{emis}}^{\text{onset}}$ (nm)	ϵ ($\text{M}^{-1}\text{cm}^{-1}$)	Φ_{F} (%)	Molecular brightness ($\text{M}^{-1}\text{cm}^{-1}$)
SiRos1300	CH_2Cl_2	1,140	1,300	1,800	115,000	0.0056 ± 0.0007	6.4
	CH_3CN	1,116	—	—	45,000	—	—
	D_2O^*	1,117	1,241	1,800	35,000	0.0068 ± 0.0009	2.4
SiRos1550	CH_2Cl_2	1,348	1,557	2,000	122,000	0.0025 ± 0.0003	3.1
	CH_3CN	1,293	—	—	61,000	—	—
	D_2O^*	1,211	1,475	>1,900	51,000	0.0021 ± 0.0003	1.1
SiRos1700	CH_2Cl_2	1,440	-1,700	2,200	98,000	0.0011 ± 0.0003	1.1
	CH_3CN	1,280	—	—	54,000	—	—
	D_2O^*	1,053	1,500	2,200	59,000	0.0007 ± 0.0001	0.4
ICG ⁵⁷	Ethanol	787	818	-1,000	194,000	13.2 ± 0.8	25,600
FD-1029 ⁵⁸	Ethanol	981	1,022	—	186,000	0.58	1,055
VIX-4 ⁹	CH_2Cl_2	1,014	1,210	-1,700	219,000	0.008 ± 0.0009	18
Flav ^{7,38,54}	CH_2Cl_2	1,027	1,053	-1,300	241,000	0.61 ± 0.02	1,470
IR-1061 ^{38,59}	CH_2Cl_2	1,063	1,081	-1,400	325,000	0.32 ± 0.04	1,040
IR-26 ³⁷	$\text{C}_2\text{H}_4\text{Cl}_2$	1,084	1,144	-1,450	130,000	0.05 ± 0.003	65
7-DMA-RI ²³	Toluene	1,096	1,256	-1,600	71,000	0.005	4
NIR1380 ²⁹	CH_2Cl_2	1,270	1,380	1,700	—	0.14	—
HC1376 ³⁰	$\text{C}_2\text{H}_4\text{Cl}_2$	1,312	1,376	-1,700	91,880	0.011	10.1

*Inside of a DSPE-mPEG2000 micelle. Determination of the quantum yield error is discussed in the Methods.

lower molar absorptivity, shift towards higher energy and broadening of the absorption features indicate that the dyes may begin to transition from a $\pi \rightarrow \pi^*$ system to a $n \rightarrow \pi^*$ charge transfer system as the solvent polarity increases. The absorptions of the dyes are also studied while inside of a DSPE-mPEG2000 micelle in D_2O solution (due to the longer-wavelength absorption of D_2O relative to H_2O)³⁶ to observe how the dyes absorb light inside of a mimicked aqueous micellar environment (Supplementary Fig. 3). Similar to the absorption spectra of the dyes in CH_3CN , the absorption spectra of the dyes in the micelle are observed to broaden and decrease in intensity compared with CH_2Cl_2 . The broadened absorption in a micellular- D_2O environment may be due to aggregate formation or increased solvent polarity.

The emission spectra and fluorescent quantum yields (Φ_{F}) of **SiRos1300**, **SiRos1550** and **SiRos1700** were measured in CH_2Cl_2 (Fig. 3, Table 1 and Supplementary Figs. 5–7). The dyes have peak emission values ($\lambda_{\text{emis}}^{\text{max}}$) of 1,300 nm, 1,557 nm and -1,700 nm for **SiRos1300**, **SiRos1550** and **SiRos1700**, respectively. All of the dyes have emission onsets ($\lambda_{\text{emis}}^{\text{onset}}$) within the ESWIR region: 1,800 nm, 2,000 nm and 2,200 nm for **SiRos1300**, **SiRos1550** and **SiRos1700**, respectively. Currently, the lowest-energy $\lambda_{\text{emis}}^{\text{max}}$ value for an organic small molecule is observed at 1,380 nm to the best of our knowledge (ref. 29), which **SiRos1550** and **SiRos1700** both surpass by -170 nm and -320 nm, respectively. The emission spectra of the materials in CH_2Cl_2 generally mirror the absorption spectra, demonstrating that the shoulder features seen in the absorption spectra are vibronic in nature. The emission spectra in CH_2Cl_2 also demonstrate sharp decreases in emission intensity in several places, the most prominent of which occurs at 1,700 nm (Fig. 3 and Supplementary Figs. 5–7). These sharp decreases in emission intensity align with the absorption spectrum of CH_2Cl_2 and are attributed to solvent reabsorption, as has been seen previously for SWIR-emitting materials (Supplementary Figs. 5–7)³⁷. Herein, solvent reabsorption corrections were performed as outlined in the Methods to bring clarity to the emission spectra (Supplementary Fig. 8–10). The Φ_{F} of **SiRos1300** was found to be $0.0056 \pm 0.0007\%$ in CH_2Cl_2 (referenced with respect to an IR-1061 Φ_{F} of $0.32 \pm 0.04\%$ in

Table 2 | Photoluminescent lifetimes, Stokes shifts, absorption and emission oscillator strengths, transition dipole moments, and degeneracy ratios of SiRos fluorophores

	Lifetime (ps)	E_{Stokes} (cm^{-1})	f_{12}	f_{21}	μ_{21} (D)	g_2/g_1
SiRos1300	20	1,080	1.14	-0.028	3.3	41
SiRos1550	26	996	1.15	-0.014	2.5	84
SiRos1700	47	1,052	1.07	-0.004	1.4	270

CH_2Cl_2) using equations (4) and (5) in the Methods³⁸. The Φ_{F} of IR-1061 was selected as the standard due to its thorough characterization in the literature, where several methods were used to show the wide variation of Φ_{F} reference values obtainable for this dye³⁸. One of the most common reference dyes (IR-26) in the SWIR has Φ_{F} reference values that range by an order of magnitude in the literature; thus, comparing Φ_{F} values between papers requires care^{17,39}. All other dyes in the series were then referenced with respect to **SiRos1300** in CH_2Cl_2 (Table 1). **SiRos1550** was measured to have a Φ_{F} of $0.0025 \pm 0.0003\%$ (roughly half that of **SiRos1300**), whereas **SiRos1700** was measured to have a Φ_{F} of $0.0011 \pm 0.0003\%$ (roughly half that of **SiRos1550**). The Φ_{F} values found herein are for the raw emission spectra and would increase slightly after solvent-reabsorption corrections because both **SiRos1550** and **SiRos1700** have considerable portions of their integrated emission intensity missing due to solvent reabsorption (Supplementary Figs. 5–7). Emission spectra were also collected inside of a DSPE-mPEG2000 micelle in D_2O to understand the molecular brightness of the dyes in an aqueous micellular environment (Supplementary Fig. 4). D_2O was selected as a solvent because it absorbs light at -300 nm longer wavelengths than H_2O (refs. 36,40). The values of Φ_{F} inside of the micelle were observed to be $0.0068 \pm 0.0009\%$, $0.0021 \pm 0.0003\%$ and $0.0007 \pm 0.0001\%$ for **SiRos1300**, **SiRos1550** and **SiRos1700**, respectively. It should be stressed that there are no organic small-molecule

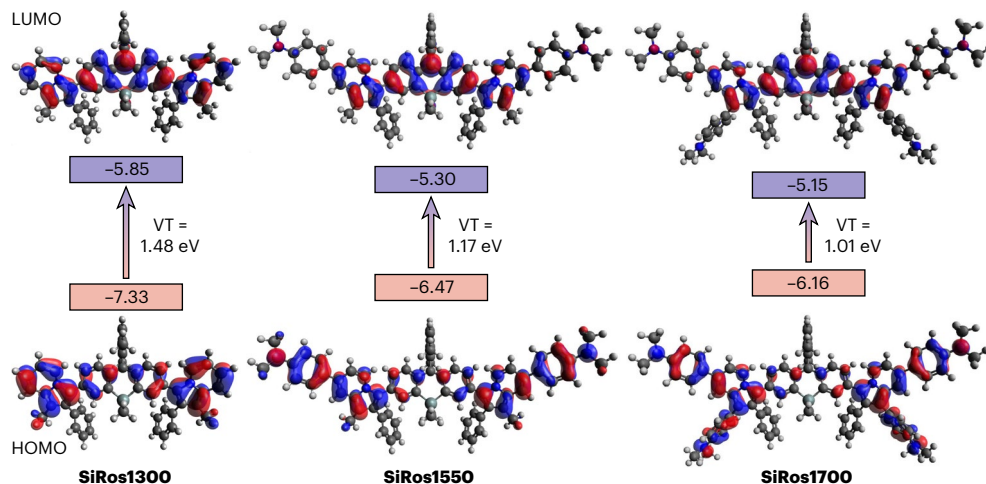


Fig. 4 | Frontier molecular orbital analysis of the SiRos fluorophores. HOMO (bottom) and LUMO (top) orbital density plots of **SiRos1300**, **SiRos1550** and **SiRos1700**, computed at the B3LYP/6-311 G(d,p) level of theory using CH₂Cl₂ as an implicit solvent. Computed energies of the HOMO and LUMO orbitals and the vertical transitions (VT) are provided along with each fluorophore.

materials that emit in this spectral region (>1,400 nm) to the best of our knowledge and that the signal-to-noise ratio in the SWIR is heavily wavelength dependent, making lower-energy emission more valuable than the sacrifice of signal intensity⁴¹.

We observed $\lambda_{\text{emis}}^{\text{max}}$ of 1,241 nm, 1,475 nm and 1,500 nm for **SiRos1300**, **SiRos1550** and **SiRos1700** in D₂O, respectively (Table 1 and Supplementary Fig. 4). Once again, the emission spectra of both **SiRos1550** and **SiRos1700** surpass the previous lowest energy observed $\lambda_{\text{emis}}^{\text{max}}$ (in CH₂Cl₂) by -95 and -120 nm, respectively. The emission spectra of **SiRos1300**, **SiRos1550** and **SiRos1700** in D₂O do not mirror the absorption spectra, which is probably due to the non-emissive aggregate states of the dyes being present in the micelle that are responsible for light absorption but not fluorescence emission. The emission spectrum of **SiRos1550** and **SiRos1700** seem to cut off sharply in D₂O at -1,900 nm due to the strong absorption feature of D₂O at 1,900 nm (Supplementary Figs. 11–13). The $\lambda_{\text{emis}}^{\text{onset}}$ values in D₂O are similar to those observed in CH₂Cl₂ for **SiRos1300** and **SiRos1700** at 1,800 nm and 2,200 nm, respectively; however, the $\lambda_{\text{emis}}^{\text{onset}}$ of **SiRos1550** is not observable due to the absorption of the D₂O solvent and is estimated herein to be >1,900 nm. Although the $\lambda_{\text{emis}}^{\text{onset}}$ of **SiRos1700** is similarly impacted by the absorption of D₂O, it demonstrates observable emission intensity past the D₂O absorption feature, revealing the true emission onset to be 2,200 nm. Overall, all three dyes demonstrate appreciable emission in the SWIR in both organic solvent and aqueous micelles and show promise as SWIR organic small molecule imaging materials.

Molecular brightness (MB = $\epsilon \times \Phi_f$) is defined as the product of the molar absorptivity and the fluorescence quantum yield of a material; it is a useful metric for comparing the relative brightness of fluorophores as it accounts for both how much light a fluorophore absorbs and how efficiently it subsequently emits that light. Molecular brightness values of 6.4 M⁻¹ cm⁻¹, 3.1 M⁻¹ cm⁻¹ and 1.1 M⁻¹ cm⁻¹ were observed for **SiRos1300**, **SiRos1550** and **SiRos1700**, respectively, in CH₂Cl₂ (Table 1). These values are similar to those observed for other SWIR-emitting xanthene fluorophores⁹ such as VIX-4 when the large bathochromic shifts of the SiRos materials are considered.

Photoluminescent lifetimes

Photoluminescent lifetimes of the SiRos dyes were determined to further understand the photophysical characteristics and excited-state kinetics of the materials (Table 2 and Supplementary Fig. 17). Upon excitation with a 785 nm laser, emitted photons were detected and timed using superconducting nanowire single-photon detectors (which are

sensitive to light from 800–2,500 nm) with >30% quantum efficiency and sub 100 ps instrument response⁴². Interestingly, the lifetimes were observed to be inversely proportional to the emission energy of the fluorophores, with **SiRos1300** having the shortest deconvolved lifetime of 20 ps, and **SiRos1700** having the longest lifetime of 47 ps. These results are counter-intuitive to previous observations of bathochromic shifts in fluorophores coinciding with increased non-radiative decay rates due to the energy gap law^{41,43–46}. There is no trend in Stokes shift (E_{Stokes}), indicating similar coupling to vibrational modes between chromophores. Furthermore, solvent reabsorption is known to decrease lifetimes due to direct energy transfer from chromophore to solvent vibrational modes⁴⁷. Thus, the lengthening of lifetimes at longer wavelengths is even more surprising given these mitigating factors.

Combining Φ_f , lifetime and absorption spectrum information, we observed that the radiative rate decreases from **SiRos1300** to **SiRos1700** ($2.8 \times 10^6 \text{ s}^{-1}$ and $2.34 \times 10^5 \text{ s}^{-1}$, respectively), whereas non-radiative rates only slightly decrease ($5 \times 10^{10} \text{ s}^{-1}$ and $2.13 \times 10^{10} \text{ s}^{-1}$, respectively). The change in Φ_f is therefore almost entirely attributable to changes in the radiative rates (k_r). Indeed, the measured transition dipole moment (μ_{21}) and the absolute value of oscillator strength of emission (f_{21}) decreases as the chromophores undergo a bathochromic shift, with μ_{21} values decreasing from 3.3 to 1.4 D, and f_{21} values decreasing from -0.028 to -0.004, through applications of radiative rate law (equations (1) and (2)) below:

$$\mu_{21} = \sqrt{\frac{3\hbar^4 c^3}{4E_g^3 \eta}} k_r \quad (1)$$

and

$$f_{21} = -\frac{2\pi\epsilon_0 m \hbar^2 c^3}{qE_g^2 \eta} k_r \quad (2)$$

Where E_g is the energy gap defined as the midpoint between the peak absorption and emission energy, q is the charge of an electron, m is the mass of an electron, ϵ_0 is the permittivity of free space and η is the refractive index. The oscillator strength (f_{12}) is then estimated by integrating the molar absorption coefficient $\int_{s_1} \epsilon(E) dE$ consistent with a single electronic state contributing to the absorption spectrum (equation (3)).

$$f_{12} = \frac{2\epsilon_0 m c \eta}{\pi \hbar q^2} \frac{1,000}{N_A \log_{10} e} \int_{s_1} \epsilon(E) dE \quad (3)$$

675 nm for **SiRos1550**, and 917 nm and 648 nm for **SiRos1700** (Supplementary Table 4). These transitions are noticeable in the absorption spectrum of the dyes in CH_2Cl_2 as the lowest energy feature of **SiRos1700** is broadened, which is consistent with the computationally predicted transition at 917 nm. **SiRos1700** also has a broad feature near 700 nm, which is consistent with the computationally predicted transition at 648 nm. Similarly, **SiRos1550** has a transition around 800 nm that is consistent with the computationally predicted transition at 675 nm.

Fluorophore chemical stability

Dye stability towards a variety of nucleophiles was studied with **SiRos1300** in CH_3CN solution by measuring the remaining absorption of the dye at regular time intervals for each condition (Supplementary Fig. 19). The absorption of **SiRos1300** was observed to rapidly decay, with a complete loss of the fluorophore absorption signal before a measurement could be taken in the presence of small anionic nucleophiles (present at $10\times$ concentration with respect to **SiRos1300**) including OH^- (K^+ counter ion), F^- (Bu_4N^+ counter ion) and CN^- (Bu_4N^+ counter ion; see Methods for experimental details). In a 10% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ solution stored under ambient conditions, **SiRos1300** was observed to maintain ~85% of its initial absorption over the course of a week, which indicates that anionic nucleophiles promote faster decay of the fluorophore absorption signal compared to neutral nucleophiles. This coincides with the reasonable stability of **SiRos1300** in large quantities of H_2O , but nearly instantaneous decay of the absorption signal in the presence of the KOH.

In vivo imaging experiments

Canola-oil-based nanoemulsions were investigated to allow for prolonged in vivo imaging. Nanoemulsions were made by emulsifying canola oil using a Pluronic F-68 surfactant in phosphate-buffered saline (Fig. 5a)⁵³. The nanoemulsions were subsequently loaded with **SiRos1300** or **SiRos1550** dissolved in acetone by rocking the mixture for at least 1 h. Note that **SiRos1700** was excluded from the imaging experiments as the range of the SWIR camera utilized herein does not capture the bulk of the emission. The SWIR-emissive nanoemulsions were washed via three successive spins through a centrifugal filter (molecular weight cut-off = 10,000 Da, 4,000 r.p.m. or $3,739\times g$), and then characterized by absorption spectroscopy, SWIR camera emission and dynamic light scattering (Fig. 5b and Supplementary Fig. 21) before in vivo use. **SiRos1300** nanoemulsions were an average size of 260 nm with a polydispersity index of 0.44, whereas **SiRos1550** nanoemulsions were an average size of 194 nm with a polydispersity index of 0.27. Absorption spectra of the **SiRos1300**- and **SiRos1550**-loaded nanoemulsions demonstrate very similar features to the absorption of the dyes in organic solvent (Fig. 3), indicating single molecule behaviour of the fluorophores in the nanoemulsions (Fig. 5b, top). **SiRos1300** nanoemulsions were observed to be roughly twice as bright as **SiRos1550** nanoemulsions under the same imaging conditions (Fig. 5b, bottom).

SiRos1300 and **SiRos1550** nanoemulsions both demonstrate excellent spatial resolution, a characteristic feature in SWIR fluorescence imaging (Fig. 5c,d and Supplementary Fig. 22). Both nanoemulsions are observed to distribute throughout the entire circulatory system shortly after tail vein injection, with well-defined resolution of the mouse vasculature in regions including the abdomen, jugular vein and femoral artery. The cross-sectional intensities are shown in Fig. 5c,d for **SiRos1300** and **SiRos1550**, respectively. **SiRos1300** is observed to produce greater peak signal intensities than **SiRos1550** along the jugular (-120 a.u. versus -100 a.u.) and femoral artery (-120 a.u. versus -70 a.u.) cross-sections, whereas both fluorophores produce comparable peak signal intensities along the abdominal cross-section (-80 au). Furthermore, mice injected

with both nanoemulsions displayed a detectable signal two weeks after injection.

With their low-energy emission, **SiRos1300**, **SiRos1550** and **SiRos1700** are primed to capitalize on the advantages of the SWIR region including: (1) imaging in the high-contrast regions of 1,400–1,600 nm; (2) imaging at greater depths; and (3) expanding multiplexed capabilities. We have demonstrated the potential for each of these avenues through capillary imaging experiments with **SiRos1300** and **SiRos1550**. Both **SiRos1300** and **SiRos1550** display robust signal when imaging with 1,400 nm and 1,500 nm longpass filters (Supplementary Fig. 23). To evaluate the impact of using low-energy wavelengths on depth penetration, we equalized the brightness of **SiRos1300** and **SiRos1550**, and three other fluorophores with higher-energy $\lambda_{\text{emis}}^{\text{max}}$: JuloChrom5 (872 nm), Chrom7 (996 nm), JuloFlav7 (1,088 nm)⁵⁴. We imaged these five capillaries at varying depths of 1% Intralipid—a common medium used as a tissue phantom (Extended Data Fig. 1; Supplementary Figs. 24 and 25)⁵⁵. Capillaries with longer-wavelength-emitting dyes can be resolved at deeper depths. Using a 1,400 nm longpass filter, only **SiRos1550** can be resolved at 4 mm depth, with **SiRos1300**, JuloFlav7, Chrom7 and JuloChrom5 being resolved at 3.5 mm, 3 mm, 2.5 mm and 2 mm, respectively. Similar findings are seen using a 1,500 nm longpass filter, with only **SiRos1300** and **SiRos1550** being resolvable at 4 mm depth. This is consistent with Intralipid experiments of previous works demonstrating superior depth penetration and resolution using longer-wavelength emitters⁵⁶. Finally, an important advantage of the SWIR region is expanding the spectral window applicable for imaging through tissue, facilitating multicolour experiments. The SiRos dyes expand to regions previously unreachable by biocompatible fluorophores. **SiRos1300** is able to be multiplexed alongside JuloChrom5 and Chrom7 (Supplementary Fig. 26). Due to limitations in excitation lasers, we were unable to increase the number of channels used for multiplexing, yet this should be able to be achieved with technology advances. The SiRos dyes demonstrate the importance of the interplay between technology and probe development, with these dyes surpassing the optimized excitation and emission ranges of current SWIR imaging set-ups.

Conclusion

Here we design and synthesize a series of SiRos fluorophores and characterize their photophysical properties. **SiRos1300**, **SiRos1550** and **SiRos1700** have $\lambda_{\text{emis}}^{\text{max}}$ of 1,300 nm, 1,557 nm and 1,700 nm, along with fluorescence quantum yields of 0.0056%, 0.0025% and 0.0011%, respectively (all in CH_2Cl_2). Photoluminescent lifetimes in the picosecond regime were observed for the series, along with an inverse trend in lifetime and emission energy, in which **SiRos1700** > **SiRos1550** > **SiRos1300**. The observed photoluminescent lifetime trend is attributed to potential mixing of the singlet and triplet excited states as the optical gap of the fluorophores decreases. Time-dependent density functional theory analysis of the fluorophores matches trends observed in the dye series in which **SiRos1700** > **SiRos1550** > **SiRos1300** for absorption wavelength and vertical transition. The frontier molecular orbitals indicate partial charge transfer behaviour, which is observed experimentally as the absorption width broadens and the molar absorptivity decreases when switching from non-polar aprotic (CH_2Cl_2) to polar aprotic solvents (CH_3CN). In vivo SWIR imaging experiments were conducted using **SiRos1300** and **SiRos1550** in canola oil-based nanoemulsions. The formulations exhibited full circulatory distribution and high-resolution imaging of mouse vasculature in the femoral arteries, abdominal cavity and jugular veins. Overall, this work illustrates design principles for future long wavelength emitting SWIR fluorophores. Future efforts are focused on designing water soluble fluorophores and higher quantum yield fluorophores in the ESIR region. Furthermore, fluorophores emitting in the midwave infrared (3,000–5,000 nm) region are being pursued based on these design principles.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41557-024-01464-6>.

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Methods

General synthetic information

We purchased 2-bromo-4-chloro-1-iodobenzene and 2-bromo-4-chlorobenzaldehyde from IPlusChem, dichlorobis(2-ethylhexyl)silane from Gelest, and DSPE-mPEG2000 from AaronChem. Common reagents and solvents were purchased from Ambeed, Tokyo Chemical Industry, Sigma-Aldrich, Acros Organics and Thermo Fischer Scientific, and were used without further purification. Thin layer chromatography was conducted using Sorbtech Silica XHL TLC plates (glass-backed support; thickness = 250 μm) and visualized with an ultraviolet lamp. Flash column chromatography was performed using a Teledyne CombiFlash RF+ system. The silica gel cartridges were purchased from Luknova SuperSep (FC003012, 50 μm).

Synthesis

Detailed synthetic procedures and characterization data are provided in the Supplementary Information.

Characterization information

^1H and ^{13}C $\{^1\text{H}\}$ NMR spectra were recorded on Bruker Ascend 300 (300 MHz) and 400 (400 MHz) spectrometers using deuterated solvents. J values are expressed in hertz and chemical shifts are in parts per million, using residual solvent as an internal standard (CDCl_3 at 7.26 ppm, CD_2Cl_2 at 5.32 ppm and $(\text{CD}_3)_2\text{CO}$ at 2.05 ppm). Singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), multiple signals (ms), broad (br) and apparent (ap) are designated as ^1H -NMR multiplicity patterns. High-resolution mass spectra were obtained with quadrupole time-of-flight, high-resolution-mass-spectrometry-utilizing nanospray ionization. The mass analyser was set to the 200–2,000 Da range. Infrared spectra were recorded with an Agilent Cary 660 attenuated total reflection-Fourier-transform infrared spectrometer. All of the absorption profiles were recorded on a Cary 5000 ultraviolet–visible–near-infrared spectrophotometer set to the double beam mode, with all dyes at a concentration of 1×10^{-5} M. All fluorescence spectra were conducted with a dye concentration of 1×10^{-5} M. The fluorescence quantum yield (referenced to IR-1061 at 0.32% in CH_2Cl_2)³⁸ of **SiRos1300** was recorded using a HORIBA QuantaMaster 8075–21 spectrofluorometer with xenon lamp excitation and a liquid-nitrogen-cooled InGaAs solid-state detector, and was determined to be 0.0056% in CH_2Cl_2 using equation (4) below:

$$\Phi_{\text{sample}} = \Phi_{\text{standard}} \times \frac{E_{\text{sample}}}{E_{\text{standard}}} \times \frac{A_{\text{standard}}}{A_{\text{sample}}} \times \frac{\eta_{\text{sample}}^2}{\eta_{\text{standard}}^2} \quad (4)$$

where E is the integrated emission counts; A is 1×10^{-4} , where the superscript A is the absorbance at the excitation wavelength; η is the refractive index of the solvent used; and Φ denotes the fluorescence quantum yield. The steady-state emission spectra shown in Fig. 3 and Supplementary Figs. 4–13 were acquired using a HORIBA Fluorolog-QM, which is the fourth generation of the Fluorolog spectrofluorometer (the set-up shown in Supplementary Fig. 1). A 980 nm diode-pumped solid-state (2 W) laser was used as the excitation source, and was mounted to the front panel of the Fluorolog-QM's sample tray. A HORIBA liquid-nitrogen-cooled solid state detector (DSS) InAs detector was used to detect the emission through a monochromator (350 mm focal length) with a ruled diffraction grating (600 grooves per millimetre, blazed at 1.25 μm), a scanning increment of 2 nm and an emission bandpass of 30 nm. To increase the detector's sensitivity, a lock-in amplifier with the liquid-nitrogen-cooled InAs detector was used. A transistor–transistor logic output signal (27 Hz) from the chopper control was used as the reference signal to the lock-in-amplifier control, and also as the trigger signal to the laser control to electrically chop the 980 nm diode-pumped solid-state laser. The time constant of the lock-in amplifier was set to 100 ms, and the detector integration time was 0.3 s. The fluorescence quantum yields of **SiRos1550**

and **SiRos1700** were subsequently referenced to the obtained value of $0.0056 \pm 0.0007\%$ for **SiRos1300**. As the equation for relative Φ_{F} (equation (4)) includes the multiplication and division of measured values, the relative standard deviation (RSD) of each Φ_{F} is calculated by taking the square root of the sum of the RSDs of each of the measured values using equation (5) below:

$$\text{RSD}_{\Phi_{\text{F}}} = \sqrt{\text{RSD}_{\Phi_{\text{R}}}^2 + \text{RSD}_{\text{AbsR}}^2 + \text{RSD}_{\text{AbsS}}^2 + \text{RSD}_{\text{EmissS}}^2 + \text{RSD}_{\text{EmissR}}^2} \quad (5)$$

The RSD for the Φ_{F} of the reference ($\text{RSD}_{\Phi_{\text{R}}}$) is well-documented in the literature to be $0.32\% \pm 0.04\%$ for IR-1061, which generates an $\text{RSD}_{\Phi_{\text{R}}}$ of 12.5%³⁸. The RSDs for the absorption values of the reference (RSD_{AbsR}) and sample (RSD_{AbsS}) were determined by taking a sample of **SiRos1300** with an absorption value comparable to those used in the emission experiments (-0.2 – 0.3), and measuring the absorption at 980 nm ten times. The absorption was observed to be 0.258 ± 0.004 , generating an RSD of 1.63%. The RSD of the emission experiments was determined by calculating the s.d. of a blank sample (s.d. = ± 0.0266 counts) and using this to determine the theoretical upper and lower bounds for the integrated emission counts. To do this, the s.d. was multiplied by the number of points collected in each emission experiment to determine how much higher (or lower) the integrated emission intensity would be if every point was 1 s.d. higher (or lower) than the measured value. The upper/lower bounds determined using this method are greater than what would likely be observed experimentally because normal, random variations in measurements typically cancel each other out; however, they provide a logical means for error propagation herein. In this way, the difference in the upper/lower bounds of emission intensity could be divided by the integrated emission intensity to obtain the RSD of the reference ($\text{RSD}_{\text{EmissR}}$) and sample ($\text{RSD}_{\text{EmissS}}$). With the RSD of each measurement determined, the RSD for each Φ_{F} experiment could be calculated and the s.d. determined (Supplementary Table 1). The recorded emission spectra were smoothed with a LOESS function, and raw data and fitted data are overlaid in Supplementary Figs. 5–7 and 11–13. Solvent selection is key for SWIR emission spectroscopy as some solvents demonstrate less and/or weaker absorption features than others, and thus have less intense dips resulting in less disrupted spectra. Although CH_2Cl_2 is not the ideal solvent for emission spectroscopy due to the intense absorption feature at 1,700 nm, it was selected due to its consistency across the literature. To correct for solvent reabsorption in the fluorescence emission spectrum of the SiRos dyes, regions of the spectra that were observed to have substantial portions of the integrated emission overlapping with solvent absorption were removed and replaced by a straight line (Supplementary Fig. 8–10). These regions included 1,300–1,550 nm, 1,580–1,780 nm and 1,800–1,950 nm. The 1,580–1,780 nm region in **SiRos1700** was replaced by straight line segments to extrapolate the peak emission for the fluorophore based on the mirror image of the absorption spectra. To do so, the x -axis of the absorption spectra was plotted in reverse energy, and the emission and absorption spectra were overlaid; points were added every 20 nm to generate a curve matching that of the absorption spectra. This was necessary to extrapolate the emission maxima of **SiRos1700**.

Photoluminescent lifetimes

Photoluminescence lifetimes for **SiRos1300**, **SiRos1550** and **SiRos1700** were recorded using a home-built, all-reflective epifluorescence set-up⁴². A pulsed 785 nm laser was used as an excitation source and the emission was collected and filtered with a 90:10 beam splitter; the appropriate excitation filters were then reflectively coupled into a single-mode fibre (F-SMF-28-C-10FC, Newport) and detected using a superconducting nanowire single-photon detector (Quantum Opus One)^{42,54,60}. Given the short photoluminescent lifetimes of the dyes, the lifetimes were fit with a convolution of the instrument response function as well as an exponential function. To determine the lifetime (or decay rate, k) for each of the time-correlated single-photon counting

traces, each curve was fit to a convolution of the sum of two gaussians with a single exponential decay as shown in equation (6) below:

$$I(t) = \frac{I_0}{2} e^{-k\left(\frac{t-t_0}{2} - \frac{\sigma_1^2 k}{2}\right)} \left(1 + \operatorname{erf}\left(\frac{(t-t_0) - \sigma_1^2 k}{\sqrt{2}\sigma_1}\right)\right) + \frac{aI_0}{2} e^{-k\left(\frac{t-t_0-t_1}{2} - \frac{\sigma_2^2 k}{2}\right)} \left(1 + \operatorname{erf}\left(\frac{(t-t_0-t_1) - \sigma_2^2 k}{\sqrt{2}\sigma_2}\right)\right) \quad (6)$$

where the width of the sum of the gaussians (σ_1 and σ_2), the time offset (t_0) between the two gaussians, and the amplitude scale (a) were determined using the instrument response function (IRF) measured as the backscatter off of a cuvette with solvent (that is CH_2Cl_2) without long-pass filters. The initial peak amplitude (I_0), the rate (k), and t_0 are free-fitting parameters, whereas the time offset (t_1) and the IRF widths (σ_1 and σ_2) were fixed variables. A conservative error of ± 1 ps (the instrument resolution) was used for the lifetimes. The IRF fit values used in the above equation are: $\sigma_1 = 40.7$, $\sigma_2 = 66.6$, $t_1 = 82.7$ and $a = 0.25$.

Fluorophore stability

All fluorophore stability studies were conducted using **SiRos1300** (2×10^{-5} M) in anhydrous CH_3CN that was freshly distilled over CaH_2 . All stability studies were conducted in triplicate to ensure reproducibility; the error bars found in the graphs are given as the s.d. of the percent-remaining absorption of the three trials at each respective time point (Supplementary Fig. 19). Nucleophiles including OH^- , F^- and CN^- were added to the **SiRos1300** solution from a stock solution to yield a final nucleophile concentration of 2×10^{-4} M ($10\times$ with respect to **SiRos1300**). The OH^- stock solution consisted of 6×10^{-2} M KOH in H_2O , and $10 \mu\text{l}$ of this solution was added to 3 ml of 2×10^{-5} M **SiRos1300** in CH_3CN to yield an OH^- concentration of 2×10^{-4} M. Identical concentrations and volumes were used for the F^- and CN^- nucleophiles with tetrabutylammonium counter ions (used to impart organic solubility), except the stock solution was made using anhydrous CH_3CN instead of H_2O . The stability of **SiRos1300** to neutral water was studied in 10% $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ as higher concentrations of H_2O were observed to impact the solubility of the hydrophobic fluorophore. The remaining absorption was tracked over the course of a week as **SiRos1300** was observed to be quite stable to neutral water. Other nucleophiles including sodium acetate (NaOAc) and *n*-butylamine were observed to have slower absorption decay rates than OH^- , F^- and CN^- yet much faster than H_2O ; $10\times$ concentration of both NaOAc and *n*-butylamine yielded relatively slow absorption decay, so higher concentrations were used to generate stability curves on shorter time scales. The concentration of saturated NaOAc in CH_3CN has previously been determined to be 1.4×10^{-3} M, $70\times$ that of **SiRos1300**, and was used as the nucleophile concentration herein⁶¹. For this, excess anhydrous NaOAc was added to a vial followed by roughly 5 ml of anhydrous CH_3CN . The solution was sonicated at 25°C for 10 min , then 3 ml of this solution was added directly to a cuvette through a syringe filter containing the necessary amount of **SiRos1300** to yield a final concentration of 2×10^{-5} M and the absorption monitored over $\sim 20 \text{ min}$; *n*-butylamine is freely soluble in CH_3CN and was used at a concentration of 2×10^{-3} M, $100\times$ with respect to **SiRos1300**; 3 ml of the *n*-butylamine solution was added directly to a cuvette containing the necessary amount of **SiRos1300** to yield a final concentration of 2×10^{-5} M and the absorption monitored over $\sim 60 \text{ min}$.

Intralipid depth experiments

Intralipid depth experiments were conducted in 1% intralipid by first matching the five dyes **SiRos1550**, **SiRos1300**, **JuloFlav7**, **Chrom7** and **JuloChrom5** in CH_2Cl_2 by brightness within 1 mm diameter capillaries using a $1,300 \text{ nm}$, $1,400 \text{ nm}$ or $1,500 \text{ nm}$ longpass filter with an exposure time of 5 ms , 10 ms or 30 ms , respectively. The 0 mm depth images were acquired using the SWIR apparatus (described below) with the capillaries placed in a non-emissive square plastic container and

submerged up to the brim with the intralipid solution. Intralipid solution was then added to the container until an additional 0.5 mm depth was formed over the capillaries, after which imaging was performed. This step was repeated up to a depth of 4 mm .

Animal procedures

Animal experiments were conducted in conformity with UCLA IACUC institutional guidelines (protocol no. ARC-2018-047). Non-invasive whole mouse imaging was performed on athymic nude female mice ($5\text{--}7$ weeks old), purchased from Charles River Laboratories. Mice were anaesthetized with inhaled isoflurane/oxygen set between 2 and 3% . Tail vein injections were performed with a catheter assembled from a 29 -gauge needle from an insulin syringe connected through plastic tubing to a syringe prefilled with isotonic saline solution. The bevel of the needle was then inserted into the tail vein and secured using glue. The plastic tubing was then connected to an insulin syringe prefilled with the nanoemulsion suspension. All probes were filtered through a $0.45 \mu\text{m}$ syringe filter prior to intravenous injection; $200 \mu\text{l}$ of each formulation was injected intravenously. After injection of all formulations, the volume of the catheter was chased with saline to ensure full dosage.

SWIR imaging apparatus

An InGaAs camera (Allied Vision Goldeye G-032 Cool TEC2) was fitted with a C-mount camera lens (Kowa LM35HC-SW) and emission filters, and mounted vertically above the imaging workspace. The camera used a sensor temperature set point of -30°C . The laser (LU1064DLD350-S70AN03; 35 W) output was coupled to a cube via a 600 nm core fibre-optic bundle (Lumics, LU_LWLO600_0720_220D1A1). The output from the fibre was fixed in an excitation cube (Thorlabs KCB1E), reflected off a mirror (Thorlabs BBE1-E03), and passed through (1) a positive achromat (Thorlabs AC254-050-AB-ML), (2) $1,300 \text{ nm}$ short-pass filters (Edmund Optics no. 84-768) and (3) an engineered diffuser (Thorlabs ED1-S20-MD) to provide uniform illumination over the working area. The excitation flux was measured over the area of interest using a digital optical power and energy meter (Thorlabs PM100D). The camera and lasers were externally controlled and synchronized by delivering trigger pulses of 5 V transistor–transistor logic to the laser drivers and camera using a programmable trigger controller. The trigger controller uses pulses generated with an Atmel Atmega328 microcontroller unit and programmed using Arduino Nano Rev 3 MCU (A000005) in the Arduino integrated development environment. The imaging data were then transferred to a PC via a gigabit ethernet interface. For image acquisition, the toolbox of MATLAB programming environment is used in combination with a MATLAB script (CCDA V3, <https://gitlab.com/brunslab/ccda>) to preview and collect the required image data in 14 -bit depth.

Image processing

Images were processed using the FIJI distribution of ImageJ. We applied laser-off background subtraction to all images (about 200 frames were taken at the beginning of every movie) and frames of interest were noted. Once the appropriate frames were chosen, the average of the frames (~ 50 frames) were obtained using the 'z-project' command. These averages were then compressed from 14 -bit ($0\text{--}14,800$ brightness) to 8 -bit ($0\text{--}255$ brightness) depth for display. These images are displayed in Supplementary Fig. 22.

Multiplexed imaging acquisition

To achieve excitation-synchronized imaging, a semi-automatic imaging algorithm implemented in MATLAB (as described above in the 'SWIR imaging apparatus' section) was combined with manual control of laser parameters through the individual laser modules and the programmed microcontroller unit. The camera captured the excitation-synchronized frames, which were then transferred to a personal computer via the GiGE interface.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The datasets generated during and/or analysed during the current study are available in a public repository <https://zenodo.org/records/10079855> and are also available from the corresponding authors on reasonable request. Further graphical data pertaining to photophysical properties, electrochemistry, photoluminescent lifetimes, computation, micelle photostability, nanoemulsions, in vivo imaging, synthetic procedures and NMR spectra are also available in the Supplementary Information. Raw data for animal experiments can be accessed on the BioImage archive #S-BIAD548. Source Data are provided with this paper.

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Author contributions

W.E.M. synthesized the intermediates and fluorophores herein and collected absorption, emission, cyclic voltammetry measurements of the fluorophores along with the computational data. E.Y.L. and I.L. conducted the in vivo and capillary imaging experiments. H.C.F. conducted the photoluminescent lifetime experiments. D.N. synthesized compound **6**. A.K.S. collected the emission spectrum and calculated the fluorescence quantum yield of **SiRos1300**. N.I.H. designed and supervised the fluorescence studies. B.Y. collected the fluorescence emission spectra for all fluorophores at HORIBA Scientific. J.R.C. designed and supervised the photoluminescent lifetime experiments. E.M.S. designed and supervised the in vivo and capillary imaging experiments. J.H.D. designed and supervised the design, synthesis and characterization of the fluorophores herein. W.E.M. wrote the initial draft of the manuscript and all authors assisted in editing subsequent drafts. All authors discussed the results and contributed to the paper.

Competing interests

W.E.M., D.N. and J.H.D. have a patent pending which includes the dyes studied herein, US Patent 20220370641A1. The remaining authors declare no competing interests.

Additional information

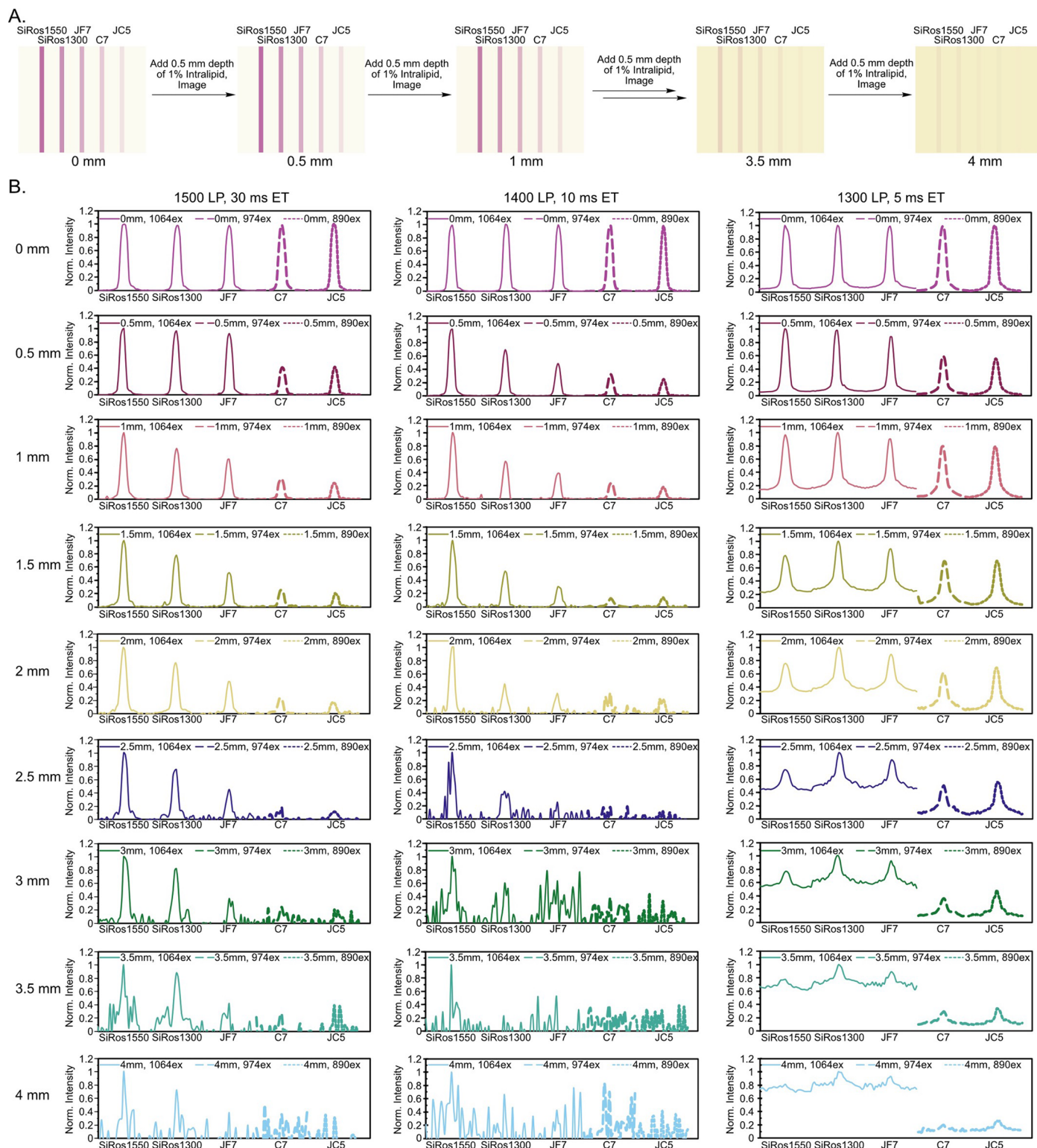
Extended data is available for this paper at <https://doi.org/10.1038/s41557-024-01464-6>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41557-024-01464-6>.

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Extended Data Fig. 1 | Evaluation and comparison of SiRos1300 and SiRos1550 depth and resolution in 1% Intralipid relative to shorter wavelength SWIR-emitting dyes. (A) Schematic of 1% Intralipid depth experiment. All dyes were initially brightness-matched in CH_2Cl_2 on the SWIR

camera and subsequently imaged at 0.5 mm depth increments using a 1,300, 1,400, or 1,500 nm LP filter. (B) Intensity plots at each 0.5 mm depth normalized to the brightest dye when using a 1,300, 1,400, or 1,500 nm LP filter. Note that the increased baseline in the 1,300 LP images is due to stray excitation light.

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Recruitment

N/A

Ethics oversight

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The sample size for the chemical stability studies was 3 independent trials for each condition.

Data exclusions

No data was excluded from the chemical stability trials.

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Each chemical stability study was conducted three separate times and all attempts showed similar results, indicating successful replication.

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Randomization was not necessary for the chemical stability studies.

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Laboratory animals

Non-invasive whole mouse imaging was performed on athymic nude female mice (5-7 weeks old), purchased from Charles River Laboratories. The mice were housed in a 12-hour light/ 12-hour dark cycle. Room temperature was set at 73F (70-76), with humidity between 30-70%

Wild animals

No wild animals were used in this study.

Reporting on sex

All mice (4 total) in this study were female. Female was arbitrarily chosen as no significant differences with respect to light penetration of tissue was expected between the two sexes. Data with respect to males have not been collected.

Field-collected samples

No field samples were collected for this study.

Ethics oversight

Animal experiments were conducted in conformity with UCLA IACUC institutional guidelines (protocol number ARC-2018-047)

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