Supplementary Information

Dual-Channel Fluorescence Imaging of Hydrogel Degradation and Tissue Regeneration in the Brain

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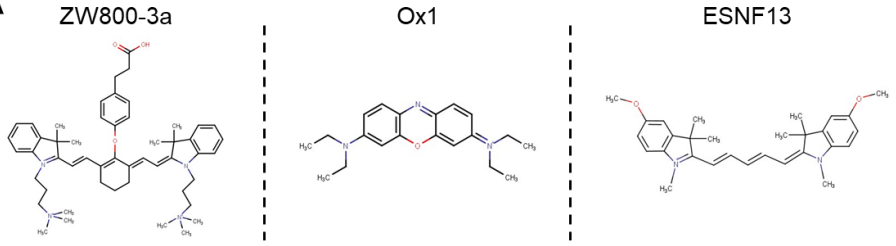
A



B

Physiochemical & Optical Properties	ZW800-3a	Ox1	ESNF13
MW	786.14	324.45	443.61
Em / Ex	774 / 790	655 / 670	674 / 700
Charges (+/-)	+3 / - 1	+1 / 0	+1 / 0
Log*D*, pH 7.4	-1.63	0.33	3.24
Acidic / Basic p*K*a	4.40 / 1.69	N/A / 3.91	N/A / 2.57
Rotatable Bonds	16	5	5
H-Bond Acceptor / Donor	4 / 1	3 / 0	3 / 0
Polar Surface Area	52.78	27.84	24.71
Extinction Coefficient ($M^{-1}cm^{-1}$)	309,000	123,000	117,000
Quantum Yield (%)	16.1	14.0	4.9

Figure S1. Physiochemical & optical properties of ZW800-3a, Ox1, and ESNF13. Physicochemical properties were calculated using JChem calculator plugins (ChemAxon, Budapest, Hungary). Optical properties were measured in FBS supplemented with 50 mM HEPES, pH 7.4 at a concentration of 1-5 µM. For QY measurements, Oxazine 725 in ethylene glycol (QY = 19%) and ICG (QY = 13%) in DMSO were used as calibration standards under conditions of matched absorbance at 660 nm (Ox1 & ESNF13) or 770 nm (ZW800-3a), respectively.

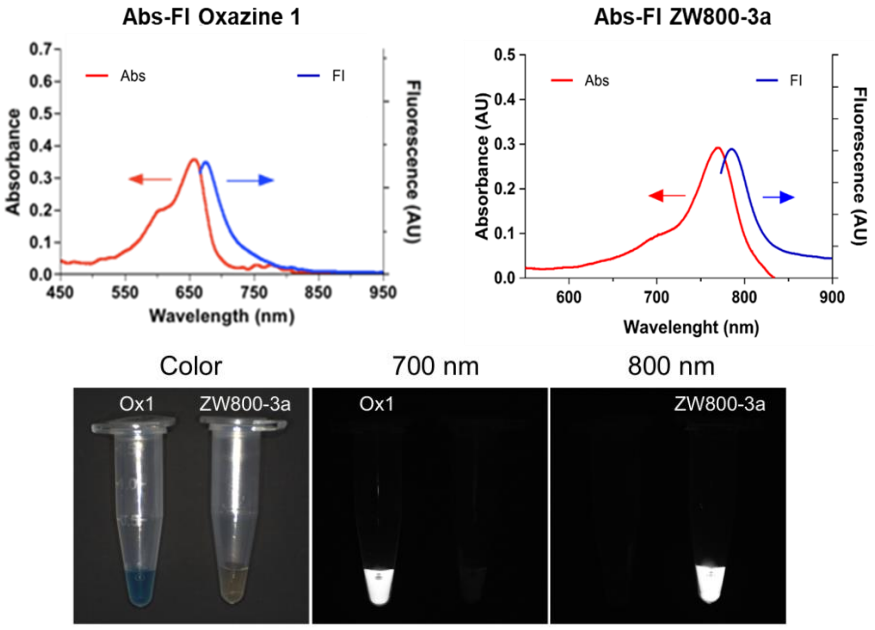


Figure S2. Optical properties of Ox1 and ZW800-3a. Absorbance and fluorescence emission spectra of each fluorophore in 10% BSA (bovine serum albumin) in PBS (5 µM) (top). Dual-channel fluorescence images of Ox1 and ZW800-3a acquired in 700 and 800 nm channels of the K-FLARE imaging system (bottom).

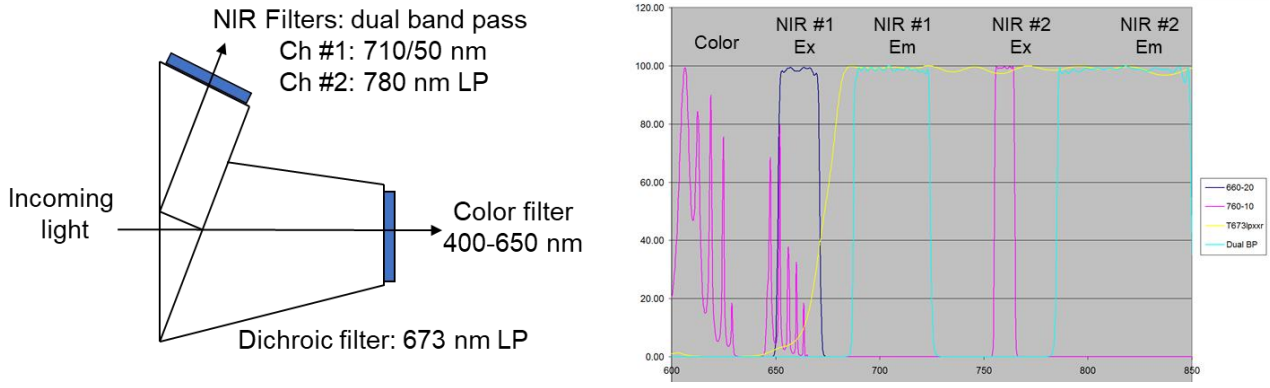


Figure S3. Optical light path and filtration for the K-FLARE imaging system. A color camera, and two intendent and simultaneous NIR camera (700 nm emission and 800 nm emission) are built in the head of the system (left). Filter profile for the color and NIR channels (right).

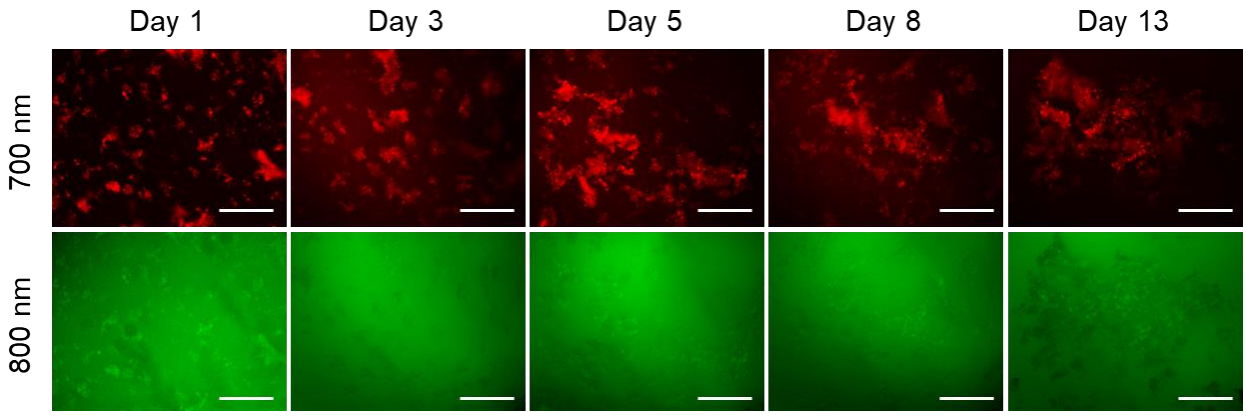


Figure S4. Live cell imaging of C2C12 embedded in the NIR hydrogel. C2C12 (700 nm) stained with ESNF13 (5 µM) were embedded in 1% NIR hydrogel (800 nm) and observed for up to 2 weeks. All images were taken at identical exposure and normalized. Scale bars = 100 µm.

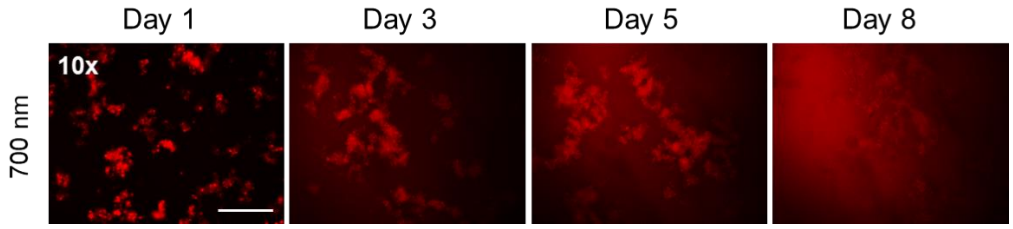


Figure S5. Live cell imaging of C2C12 embedded in 3% NIR hydrogel. C2C12 (700 nm) stained with ESNF13 (5 µM) were embedded in 3% NIR hydrogel and observed for up to 8 days. All images were taken at identical exposure and normalized. Scale bar = 100 µm.

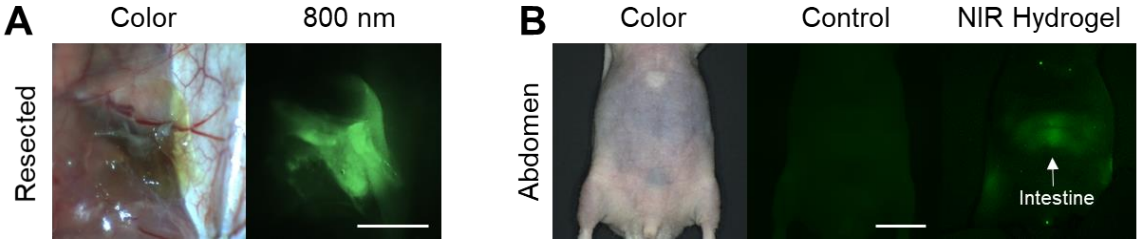


Figure S6. Biodistribution and clearance of NIR hydrogel. Long-term degradation and biodistribution of NIR hydrogel were imaged at 21 days post-implantation, compared with a control animal without scaffold implantation. Animals were fed white chow without chlorophyll to avoid autofluorescence. Scale bars = 1 cm.

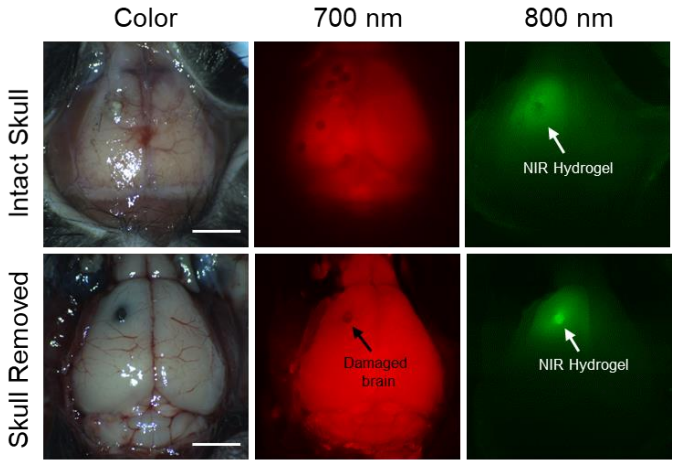


Figure S7. Dual-channel *in vivo* imaging of brain tissue growth and NIR hydrogel. NIR hydrogel (20 µL) was injected into the brain of animals and Ox1 (100 nmol) was administered to the same animal an hour prior to the dual-channel intraoperative imaging at day 7-d of post-implantation. Brain tissue (red) and NIR hydrogel (green) degradation observed with intact skull and after removing the skull. Scale bars = 2.5 mm.

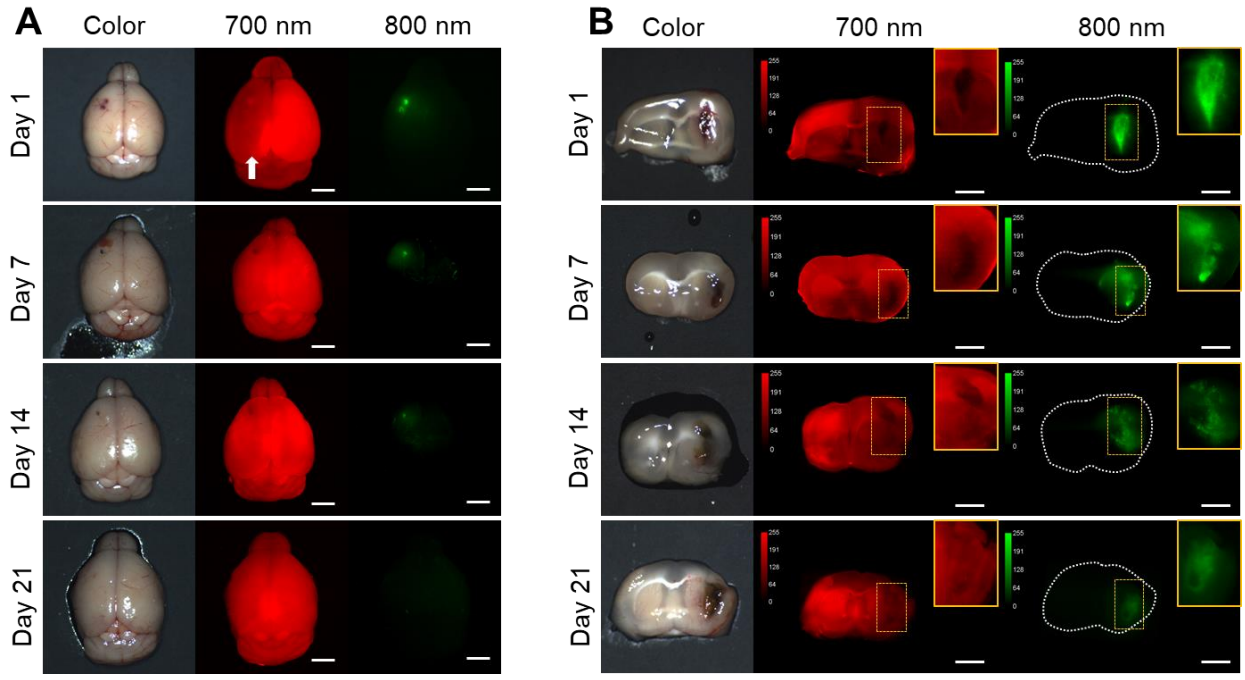


Figure S8. Dual-channel simultaneous imaging in the brain. (A) Whole brain imaging of the of Ox1 (700 nm) and NIR hydrogel (800 nm) distribution change over time. (B) Intraoperative NIR imaging of tissue ingrowth and NIR hydrogel at 700 and 800 nm channel, respectively. Sample thickness = 2 mm. Scale bars = 2.5 mm.