Electronic Supplementary Material (ESI) for Polymer Chemistry.

Investigation of two-photon polymerized microstructures using fluorescence lifetime measurements

Xingyu Wu,[a] Mehdi Belqat,[a] Benjamin Leuschel,[a] Guillaume Noirbent,[b] Fédéric Dumur,[b] Karine Mougin,[a] Arnaud Spangenberg*[a]

[a]*Institut de Science des Matériaux de Mulhouse (IS2M),*

CNRS – UMR 7361, Université de Haute Alsace,

15 rue Jean Starcky, 68057 Mulhouse, France

E-mail: arnaud.spangenberg@uha.fr

[b] *Aix Marseille Univ, CNRS, ICR, UMR 7273, F-13397 Marseille – France*

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Experimental Procedures

Materials

All chemical compounds except Bodipy-C12 were purchased from Sigma-Aldrich. Glycerol and methanol were used to prepare the formulations for the calibration of FLIM. Pentaerythritol triacrylate (PETA), poly(ethylene glycol) diacrylate 700 (PEGDA 700) and 4,4'-bis(*N,N*-diethylamino) benzophenone (DEABP) were used to prepare the formulations for two-photon fabrication. Ethanol was also used to develop and rinse the structures.

Methods and characterization

Synthesis and characterization of Bodipy-C12

Bodipy-C12 was synthesized according to the literature.[1] Below are the synthetic procedures.

4-Dodecyloxybenzaldehyde (1.16 g, 4.0 mmol) and pyrrole (11 mL, 160 mmol) were added to a round-bottom flask and degassed with bubbling of argon for 10 min. Trifluoroacetic acid (TFA, 30.6 µL, 0.4 mmol) as a catalyst was then injected, and the solution was stirred at room temperature for two hours. The solution was diluted with 50 mL chloroform, washed with 0.1 M NaOH. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel) using dichloromethane (DCM) as the eluent. 5-(4-Dodecyloxyphenyl)dipyrromethane was obtained and directly engaged in the next step.

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 280 mg, 1.23 mmol) was added to a solution of 5-(4-dodecyloxyphenyl)dipyrromethane (500 mg, 1.23 mmol) in toluene (25 mL). The reaction mixture was kept in the dark and stirred at room temperature. After 5 minutes of stirring, triethylamine (1.2 mL, 8.61 mmol) was added followed immediately by boron trifluoride etherate (1.1 mL of neat BF_3-etherate, 8.61 mmol). The reaction mixture was stirred at room temperature for 1 hour and the dark green sludge was decanted. Then, this sludge was washed with toluene (10 mL) and the combined toluene extracts were washed with water, dried (Na_2SO_4) and evaporated to give a dark-green viscous oil. Finally, the mixture was purified by column chromatography (silica gel, DCM) to give the compound as a red-orange crystalline solid (478 mg, 86% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 2H), 7.54 (d, *J* = 8.7 Hz, 2H), 7.03 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 4.1 Hz, 2H), 6.55 (d, *J* = 2.6 Hz, 2H), 4.05 (t, *J* = 6.5 Hz, 2H), 1.89 – 1.80 (m, 2H), 1.49 (dd, *J* = 15.1, 7.3 Hz, 2H), 1.43 – 1.23 (m, 16H), 0.89 (t, *J* = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.78, 147.57, 143.31, 134.84, 132.45, 131.34, 126.10, 118.23, 114.53, 68.37, 31.92, 29.66, 29.64, 29.60, 29.57, 29.37, 29.35, 29.16, 26.03, 22.69, 14.11.

^{19}F NMR (376 MHz, $CDCl_3$) δ -145.08, -145.16, -145.24, -145.32.

HRMS (ESI MS) m/z: theor: 452.2811 found: 452.2813 ($[M]^{+\cdot}$ detected).

^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra of Bodipy-C12 are shown in Figure S1-3.

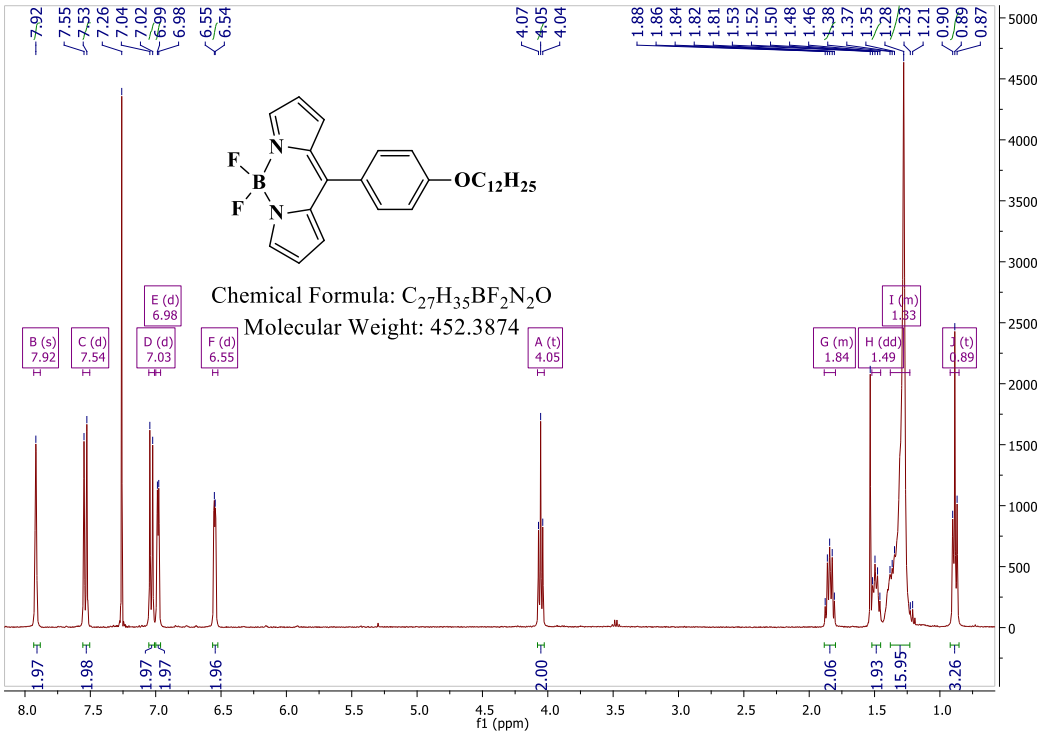

Figure S1. ^{1}H NMR spectra of Bodipy-C12 in $CDCl_3$.

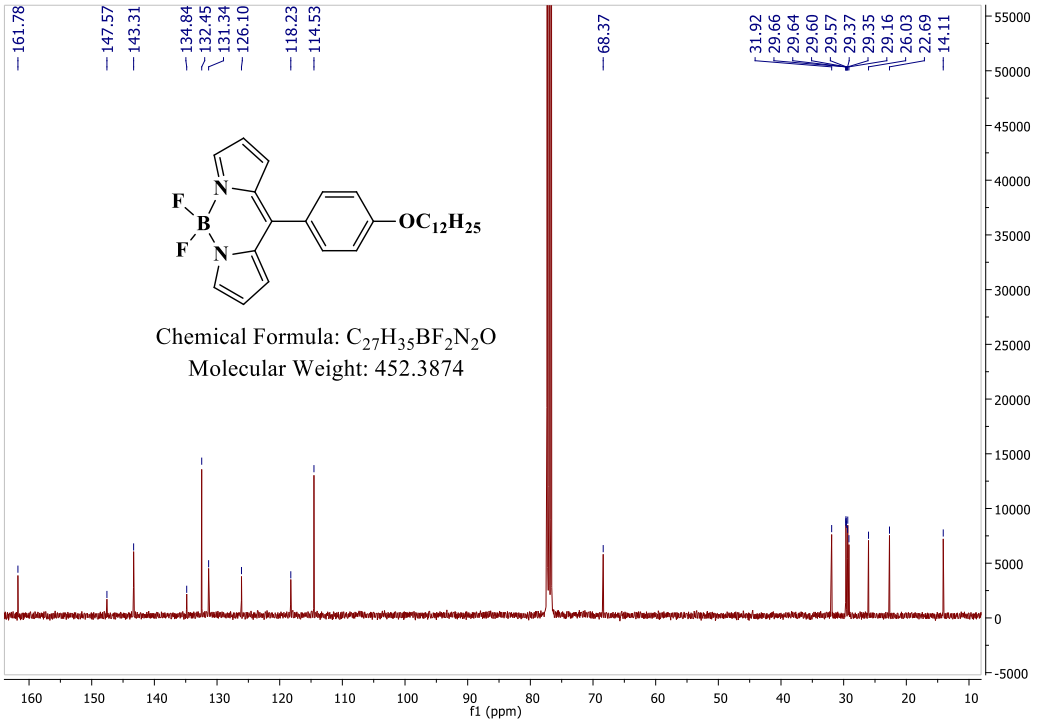

Figure S2. ^{13}C NMR spectra of Bodipy-C12 in $CDCl_3$.

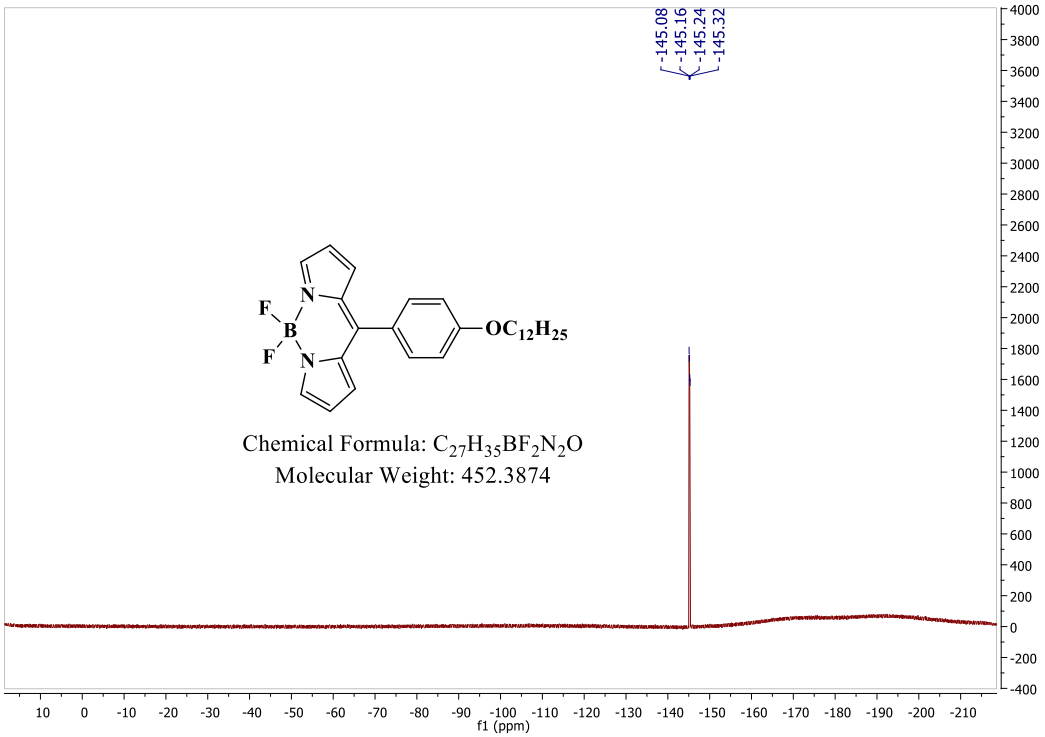

Figure S3. ^{19}F NMR spectra of Bodipy-C12 in $CDCl_3$.

Fourier transform infrared spectroscopy (FTIR)

Kinetics of photopolymerization were monitored in situ by real-time Fourier transform infrared spectroscopy (FTIR) with a Thermo-Nicolet iS-5 instrument. A drop of photocurable formulation was deposited on a silicon wafer, then covered by a transparent polypropylene film. The sample was irradiated at 365 nm using a Xe–Hg lamp (Hamamatsu, L8252, 150 W) equipped with a band pass filter. The evolution of double bond (C=C) content of PETA and PEGDA 700 was continuously followed by real time FTIR spectroscopy at around 1630 cm^{-1}. The conversion of C=C is determined by measuring the peak area of these bands and calculated with the equation [C (%) = $(A_0 – A_t)/A_0$ x 100] where C is the degree of conversion of functional groups at t time, A_0 the initial peak area before irradiation and A_t the peak area of the functional groups at t time.

Photostructuration

For all structuring, a two-photon microfabrication setup was used. It includes a standard microscope (Zeiss Observer D1), a piezo stage (PI Mars 300 μm^3), a femtosecond laser (Coherent Chameleon Ultra II, λ=800 nm, τ=140 fs, f=80 MHz), and a dedicated software to optimize fabrication paths and control the machine (SimPoly).[2] In this study, the laser beam was introduced via a 40× objective (NA: 0.65) and the exposure time was set to 10 ms with different exposure powers. After laser exposure, the samples were developed with ethanol except the non-developed samples.

Scanning electron microscope (SEM)

SEM images were obtained with a Quanta 400 FEI (Thermo Fischer Scientific).

Fluorescence lifetime imaging microscopy (FLIM) and fluorescence microspectroscopy

FLIM measurements were performed on a PicoQuant MicroTime 200 fluorescence lifetime microscope system with an Olympus IX83 inverted microscope and a water immersion objective (60x, NA 1.2). For excitation, a pulsed laser (PicoQuant LDH-D-C-485, λ=480 nm) with a repetition rate of 40 MHz and 80 ps pulse width was used. Images (512x512 pixels) were acquired using a dwell time of 30 µs per pixel. The excitation and emission signals were separated by a dichroic beamsplitter (AHF ZT470-488/640). After passing a 488 nm longpass filter and a 520/35 nm bandpass filter, the fluorescence signal was captured by a single photon sensitive detector (PicoQuant PMA Hybrid). The fluorescence signal could alternatively be redirected to a coupled spectrograph (Andor Kymera 193i with Andor Newton EMCCD Sensor) after passing the 488nm longpass filter. Analysis of FLIM data was performed using the PicoQuant SymphoTime 64 software. The experimental setup for fluorescence lifetime measurement and fluorescence microspectrometry is shown in Figure S4.

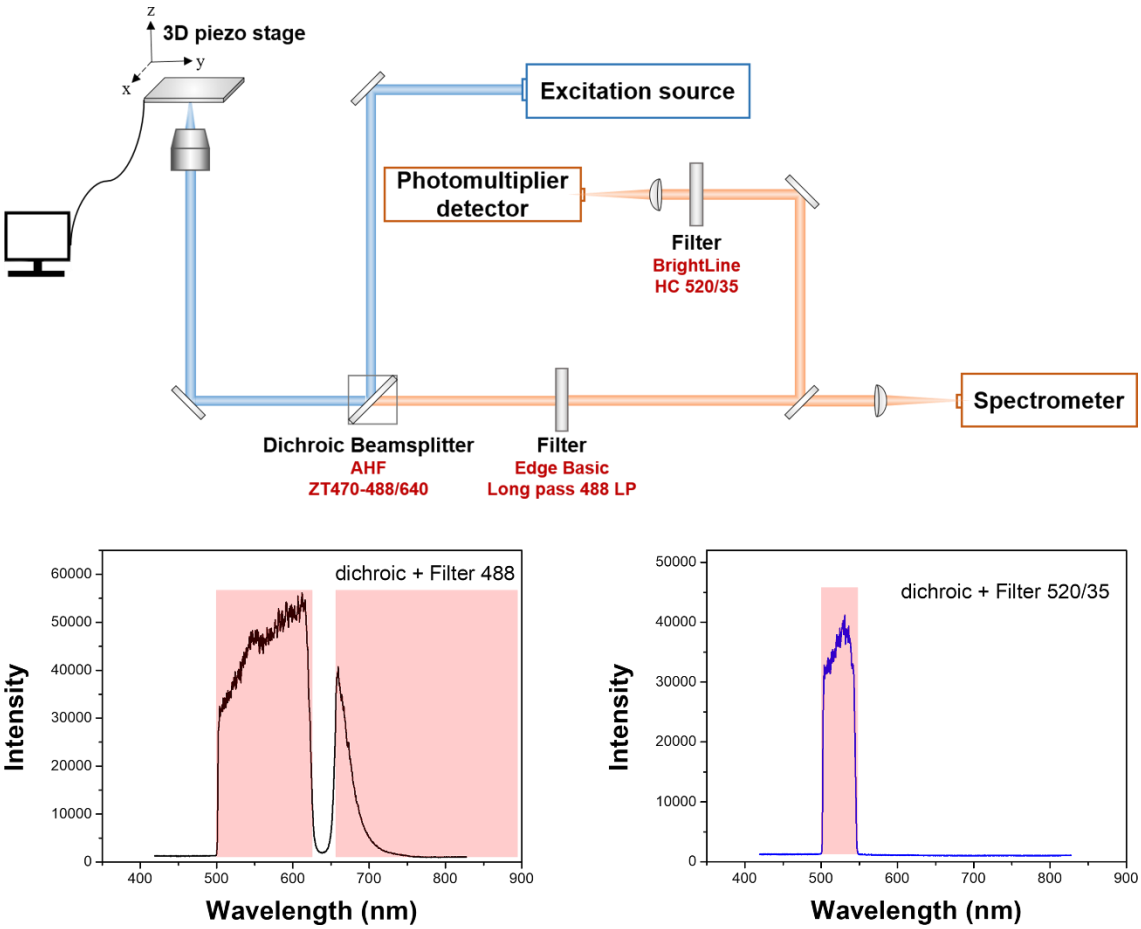

Figure S4. Top: experimental setup for fluorescence lifetime measurement and fluorescence microspectrometry. The two spectra show the transmission bands of the dichroic beamsplitter in combination with each of the two filters. Bottom left shows the transmission that applies for the fluorescence spectra measurement, bottom right corresponds to the measured wavelength band for fluorescence lifetime measurements.

Results and Discussion

Influence of Bodipy-C12 on photopolymerization

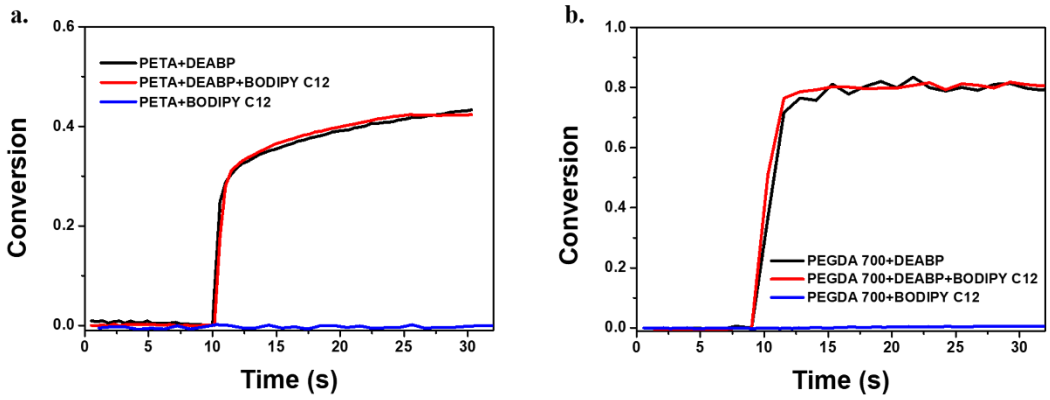

Figure S5. Evolution of double bond (C=C) conversion as a function of the irradiation time for PETA systems (a) and PEGDA 700 systems (b). In the formulation, DEABP is 1 wt% of PETA or PEGDA 700, Bodipy-C12 is 10^{-4} mol L^{-1}.

Fluorescence lifetime characterization of Bodipy-C12 in methanol/glycerol mixtures

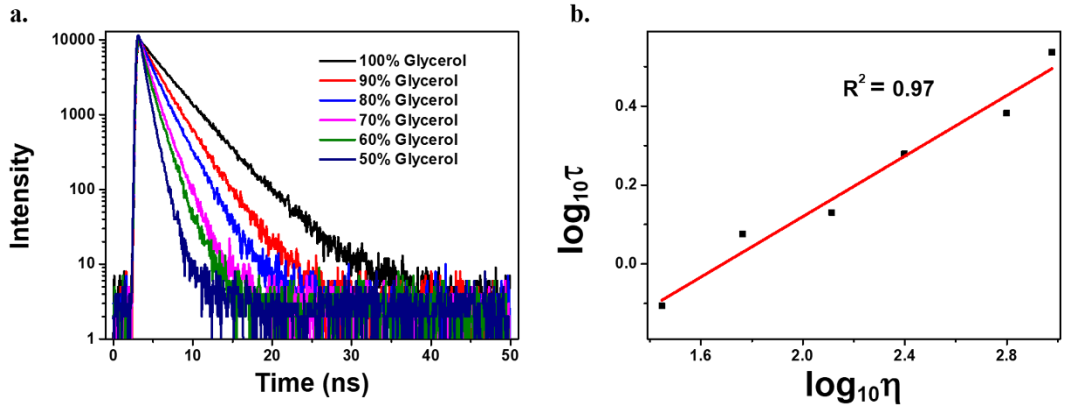

Figure S6. a. Fluorescence decay curves of Bodipy-C12 in the mixture of methanol and glycerol in volume. b. Dependency of fluorescence lifetime versus viscosity.

The fluorescence lifetime (τ) of molecular rotors as a function of viscosity (η) is described as equation (1).[3-5]

$$\tau = z k_r^{-1} \eta^{\alpha} \qquad (1)$$

Where k_r is the radiative rate constant, z and α are constants. Taking the logarithm of equation (1), a plot of $\log_{10}\tau$ versus $\log_{10}\eta$ should yield a straight line (equation S2). This can be served as a calibration.

$$log_{10}\tau = \alpha log_{10}\eta + log_{10}\frac{z}{k_r} \qquad (2)$$

The measured time-correlated single photon counting (TCSPC) curves were fitted with an exponential function (n=1 or 2, equation 3).

$$I_{(t)} = \int_{t'=0}^{t} F_{IRF}(t') \sum_{i=1}^{n} A_i \exp\left(\frac{t-t'}{\tau_i}\right) dt' \qquad (3)$$

Here, F_{IRF} is the instrument response function, A_i means the amplitude of the i_{th} component and τ_i is the lifetime.

A weighted average fluorescence lifetime was defined as below (equation 4).

$$\bar{\tau} = \frac{\sum_{i=1}^{n} A_i \cdot \tau_i}{\sum_{i=1}^{n} A_i} \qquad (4)$$

Fluorescence spectra of Bodipy-C12 in methanol, PETA, PEGDA-700 resin and microstructures

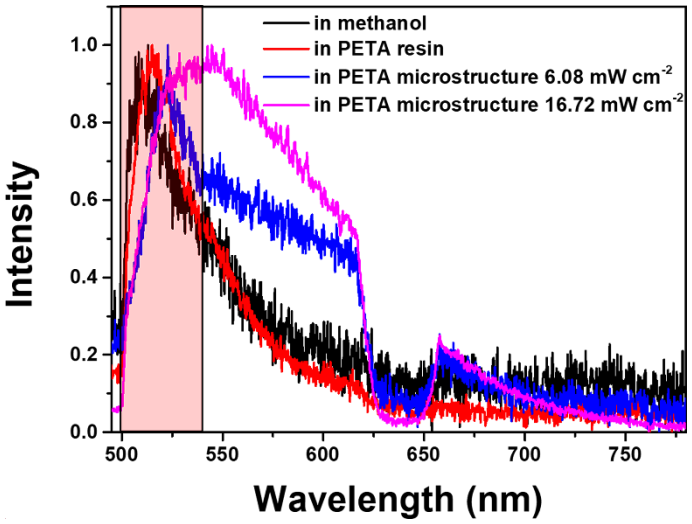

Figure S7. Fluorescence spectra of Bodipy-C12 in methanol, PETA resin and microstructures. The cherry red background is corresponding to 520/35 nm bandpass filter (see Figure S4).

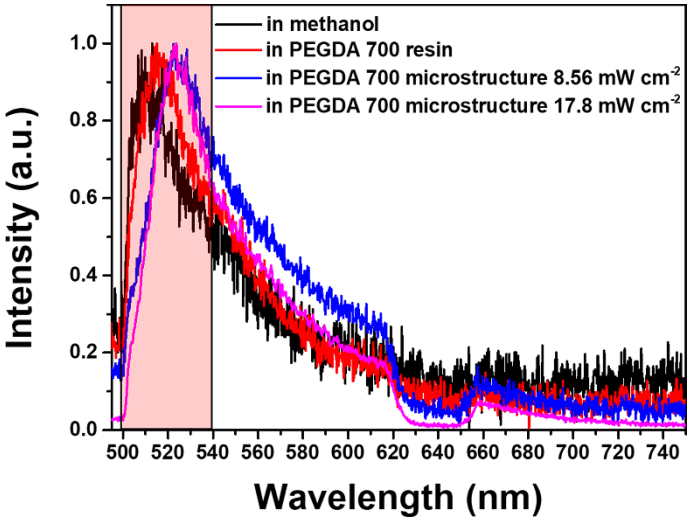

Figure S8. Fluorescence spectra of Bodipy-C12 in methanol, PEGDA 700 resin and microstructures. The cherry red background is corresponding to 520/35 nm bandpass filter (see Figure S4).

According to the fluorescence spectra, we selected the wavelength band between 500-540nm for the measurement of fluorescence lifetime.

Fluorescence lifetime characterization of Bodipy-C12 in PETA microstructures

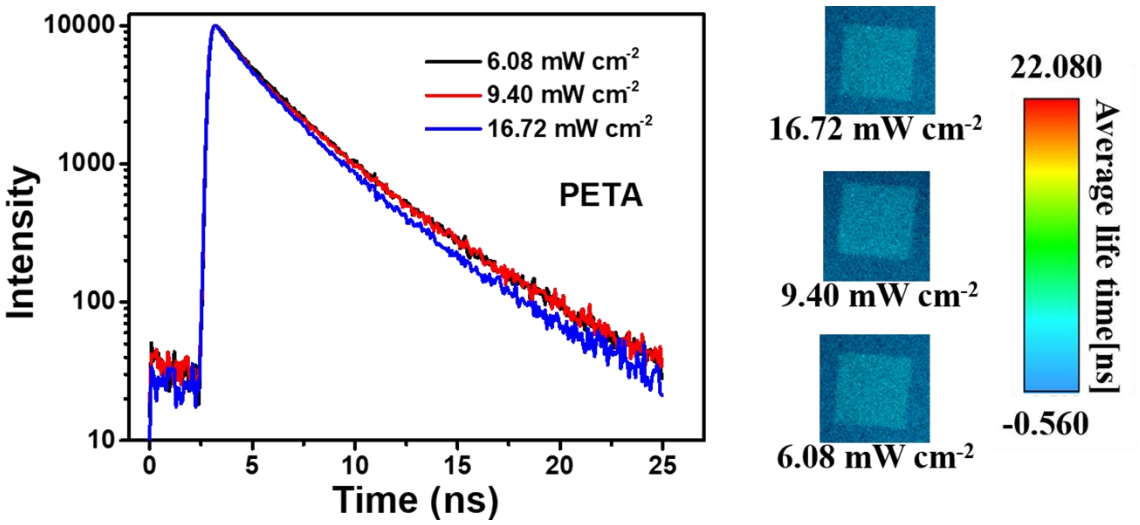

Figure S9. Fluorescence decay curves and fluorescence lifetime images of Bodipy-C12 in two-photon fabricated microstructures made from PETA at different intensities.

Table S1. Summary of Fluorescence lifetimes and amplitudes of Bodipy-C12 in PETA microstructures.

	Long lifetime (ns)	Amplitude of long lifetime	Short lifetime (ns)	Amplitude of short lifetime	weighted average fluorescence lifetime (ns)	$\chi^{2\,a}$
Undeveloped microstructures						
PETA 6.08 mW cm^{-2}	4.2 ± 0.12	0.66	1.93 ± 0.088	0.34	3 ± 0.019	0.92
PETA 9.40 mW cm^{-2}	4.0 ± 0.12	0.77	1.6 ± 0.11	0.23	2.98 ± 0.013	1.142
PETA 16.72 mW cm^{-2}	3.8 ± 0.15	0.68	1.8 ± 0.12	0.32	2.77 ± 0.022	0.915

a. χ^2 as a goodness of fit parameter. $\chi^2 \approx 1$ indicates a good fit.

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