

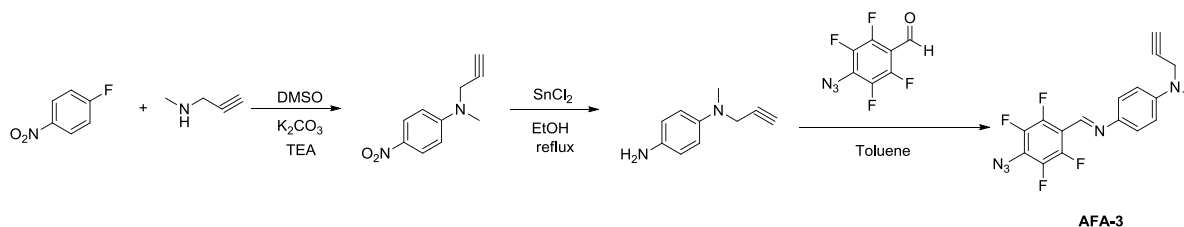
EXPERIMENTAL

Materials

All reagents for synthesis were purchased from Sigma-Aldrich, Fluka, and ABCR and were used without any further purification. The solvents were dried and purified by standard laboratory methods. Column chromatography was performed with conventional techniques on VWR silica gel 60 (0.040 – 0.063 mm particle size). Aluminum-backed silica gel plates were used for TLC analyses.

Synthesis

The novel fluoroaryl azide **AFA** was prepared as describe in Scheme 1.



Scheme 1. Reaction scheme for the synthesis of **AFA-3**

Synthesis of N-methyl-4-nitro-N-(prop-2-yn-1-yl)aniline¹

1.93 g of 4-fluoro-1-nitrobenzene (13.74 mmol), 0.95 g of N-methylpropargylamine (13.74 mmol), 2.27 g of potassium carbonate (16.48 mmol) and 1 mL of triethylamine were dissolved in 20 mL of DMSO. The mixture is stirred overnight at 50 °C and then poured into cold water. The precipitate is collected by filtration and washed with water (3 × 50 mL) and 2-propanol (20 mL) to yield the product as yellow solid. Yield: 75%. Mp: 93-95 °C. ¹H NMR (200MHz, CDCl₃) δ = 8.09 (d, *J* = 9.4 Hz, 2 H), 6.68 (d, *J* = 9.4 Hz, 2 H), 4.09 (d, *J* = 2.3 Hz, 2 H), 3.07 (s, 3 H), 2.20 (t, *J* = 2.3 Hz, 1 H). ¹³C NMR (50MHz, CDCl₃) δ = 153.1, 126.0, 111.6, 110.0, 77.7, 72.8, 42.0, 38.6

Synthesis of N¹-methyl-N¹-(prop-2-yn-1-yl)benzene-1,4-diamine

0.2 g of N-methyl-4-nitro-N-(prop-2-yn-1-yl)aniline (0.91 mmol) and 2.05 g of tin (II) chloride dihydrate (9.1 mmol) were dissolved in 30 mL of EtOH. The mixture is stirred and heated under reflux for 21 h. The cooled reaction mixture was neutralised by the addition of 5 M NaOH. The precipitate was removed by centrifugation and the solution was extracted with EtOAc. The combined organics were washed with brine and dried. The brown product was used for the next step without further purification. ¹H NMR (200MHz, CDCl₃) δ = 6.81 (d, *J* = 9.4 Hz, 2 H), 6.67 (d, *J* = 9.4 Hz, 2 H), 3.95 (d, *J* = 2.2 Hz, 2 H), 3.70 - 3.20 (bs, 2 H), 2.87 (s, 3 H), 2.18 (t, *J* = 2.2 Hz, 1 H).

Synthesis of (E)-N¹-(4-azido-2,3,5,6-tetrafluorobenzylidene)-N⁴-methyl-N⁴-(prop-2-yn-1-yl)benzene-1,4-diamine (AFA-3)

109.5 mg of 4-azido-2,3,5,6-tetrafluorobenzaldehyde (0.52 mmol) and 83.8 mg of N¹-methyl-N¹-(prop-2-yn-1-yl)benzene-1,4-diamine (0.52 mmol) were dissolved in 10 mL of dry toluene. The solution was stirred overnight under room temperature and then the solvent was evaporated under vacuum. The dark mixture was washed with dry ether to yield 93 mg (50%) of product as yellow powder. Mp: 127-129 °C (decomposed). ¹H NMR (200MHz, CDCl₃) δ = 8.54 (s, 1 H), 7.28 (d, *J* = 9.0 Hz, 2 H), 6.86 (d, *J* = 9.0 Hz, 2 H), 4.04 (d, *J* = 2.2 Hz, 2 H), 2.98 (s, 3 H), 2.15 (t, *J* = 2.2 Hz, 1 H). ¹³C NMR (50MHz, CDCl₃) δ = 148.8, 144.2 - 144.1 (m, F-C), 143.4 - 143.0 (m, F-C), 141.5, 138.4 - 137.9 (m, F-C), 122.6, 114.2, 112.3 - 111.8 (m, F-C), 78.9, 72.2, 42.4, 38.7. ¹⁹F NMR (376MHz, CDCl₃) δ = -142.9 (m, 2F), -152.4 (m, 2F). IR (cm⁻¹): 3254, 2182, 2110, 1618, 1594, 1568, 1514, 1496, 1482, 1412, 1360, 1242, 1000, 821. Anal. Calcd for C₁₇H₁₁F₄N₅: C, 56.51; H, 3.07; N, 19.38; Found: C, 56.33; H, 2.97; N, 19.55.

Open aperture Z-scan measurement

A Ti:sapphire laser system (25 fs pulse duration ,1 kHz repetition rate at 798 nm) was used for the open aperture Z-scan analysis. A detailed description of the set characterization can be found elsewhere.² **AFA-3** solution was prepared with concentration of 1.0×10^{-2} M in spectroscopic grade dimethylformamide (DMF). The sample solutions were measured in a 0.2 mm thick flow cell in a non-recycling volumetric flow of 4 mL/h. The measurements were carried out at different pulse energies ranging from 50-215 nJ. The used intensities were limited in this range since at higher intensities the solvent contributes to the effective nonlinear absorption and at lower intensities no nonlinear absorption was observed. The experimental data of **AFA-3** were fitted using the adopted equations of Sheik-Bahae et al.³ to obtain the σ_{TPA} (Fig.S1).

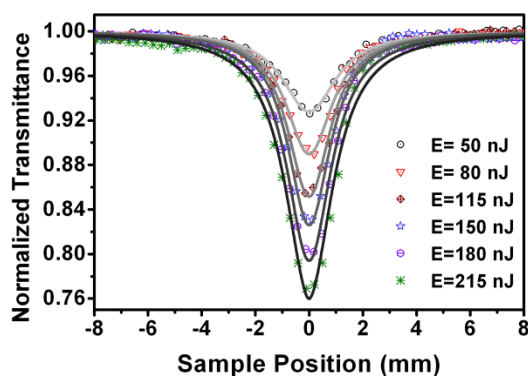


Figure S1 Experimental z-scan data (dots) and fitting curves (full lines) for **AFA-3** at different pulse energies.

Two-photon grafting

The samples were prepared from poly(ethylene glycol) diacrylate (PEGda, average M_n 700, Sigma Aldrich) and DMF mixture (ratio 50:50) with an addition of 1 wt% of the photoinitiator Irgacure 819 (Ciba Specialty Chemicals Inc.). The resulting formulation was photopolymerized with UV light for 5 min (Intelliray 600) in silicone molds (diameter 6 mm; thickness 1 mm). The photopolymerized

material pellets were soaked in DMF solution for at least 1 week, with the solvent being exchanged on a regular basis, in order to remove residual monomer and photoinitiator. For laser photografting, the samples were immersed into the 14 mmol/L solution of **AFA-3** in DMF. A Ti:sapphire femtosecond laser (Femtotrain, HighQ), emitting pulses with duration of 80 fs at a 73 MHz repetition rate around 793 nm was used for laser grafting. The laser beam was focused with a 20× microscope objective (Zeiss, NA = 0.8) into the sample. An acousto-optical modulator was used for fast switching of the laser beam and for adjusting its intensity. The laser power was measured before the objective. The patterns were obtained by scanning the focused laser beam within the sample by the galvo-scanner (HurryScan, ScanLab). In processing window tests (Fig 2a), the grafted square patterns were produced by a set of adjacent line scans at a distance of 0.5 μm. In the vertical direction a step of 5 μm (total 7 slices) was used. The lateral resolution was estimated by the single-line scan method. The lines arrays were grafted with different distance between them using the average laser power of 350 mW and scanning speed of 5 mm s⁻¹. The minimum distance between two lines when it is still possible to distinguish two adjacent lines, was found to equal to ~ 3.6 μm. After the grafting procedure, the samples were placed in DMF in order to remove the residual **AFA-3**. Rapid decoloration of the pellet indicated the successful removal of possible residuals from the sample. The fluorescence of the patterned samples was analyzed by laser scanning microscopy (LSM700 ZEN software, Carl-Zeiss) at the excitation wavelength of 555 nm. Figure S2 shows the fluorescence intensity of the produced patterns with different writing speeds and laser powers.

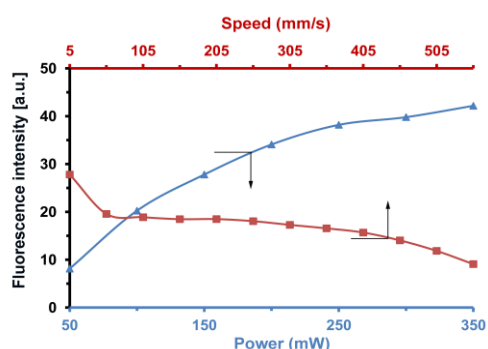
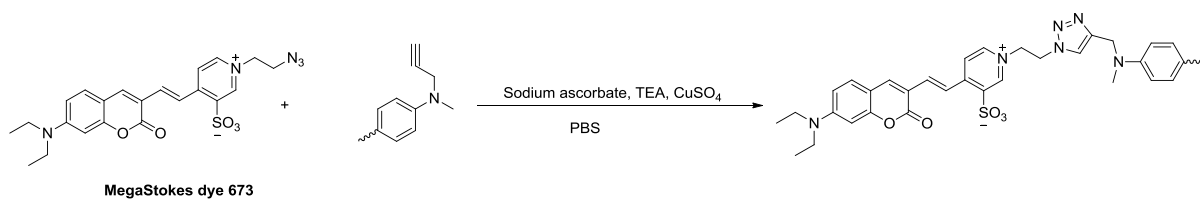


Figure S2 Fluorescence intensity of the patterns with different processing parameters: different laser powers with constant writing speed of 5 mm/s (triangle); different writing speeds with constant laser power of 150 mW (square).

CuAAC click reaction



Scheme 2. Reaction scheme for post-modification via CuAAC reaction

1 mg (2.13×10^{-3} mmol) of MegaStokes dye 673 was dissolved in 2 mL of PBS solution in a brown glass vial. To the solution, laser-grafted pellets, 0.99 mg (6.20×10^{-3} mmol) of CuSO_4 , 2.47 mg of sodium ascorbate (12.46×10^{-3} mmol) and one drop of triethylamine were added. The vial was placed on a rotation mixer after being sealed and the reaction was carried out at room temperature in dark for 12 h. Afterwards the pellet was removed, washed several times with fresh PBS and DMF to remove residual MegaStokes dye 673 and immersed into DMF solution for LSM observation.

Reference:

1. Wolfe, D. B.; Oldenburg, S. J.; Westcott, S. L.; Jackson, J. B.; Paley, M. S.; Halas, N. J. *Langmuir* **1999**, 15, (8), 2745-2748.
2. Ajami, A.; Husinsky, W.; Liska, R.; Pucher, N. *J. Opt. Soc. Am. B* **2010**, 27, (11), 2290-2297.
3. Sheik-bahae, M.; Said, A. A.; Wei, T. H.; Wu, Y. Y.; Hagan, D. J.; Soileau, M. J.; Van, S. E. *W. Proc. SPIE-Int. Soc. Opt. Eng.* **1990**, 1148, (Nonlinear Opt. Prop. Mater.), 41-51.